Irradiation to Ensure the Safety and Quality of Prepared Meals

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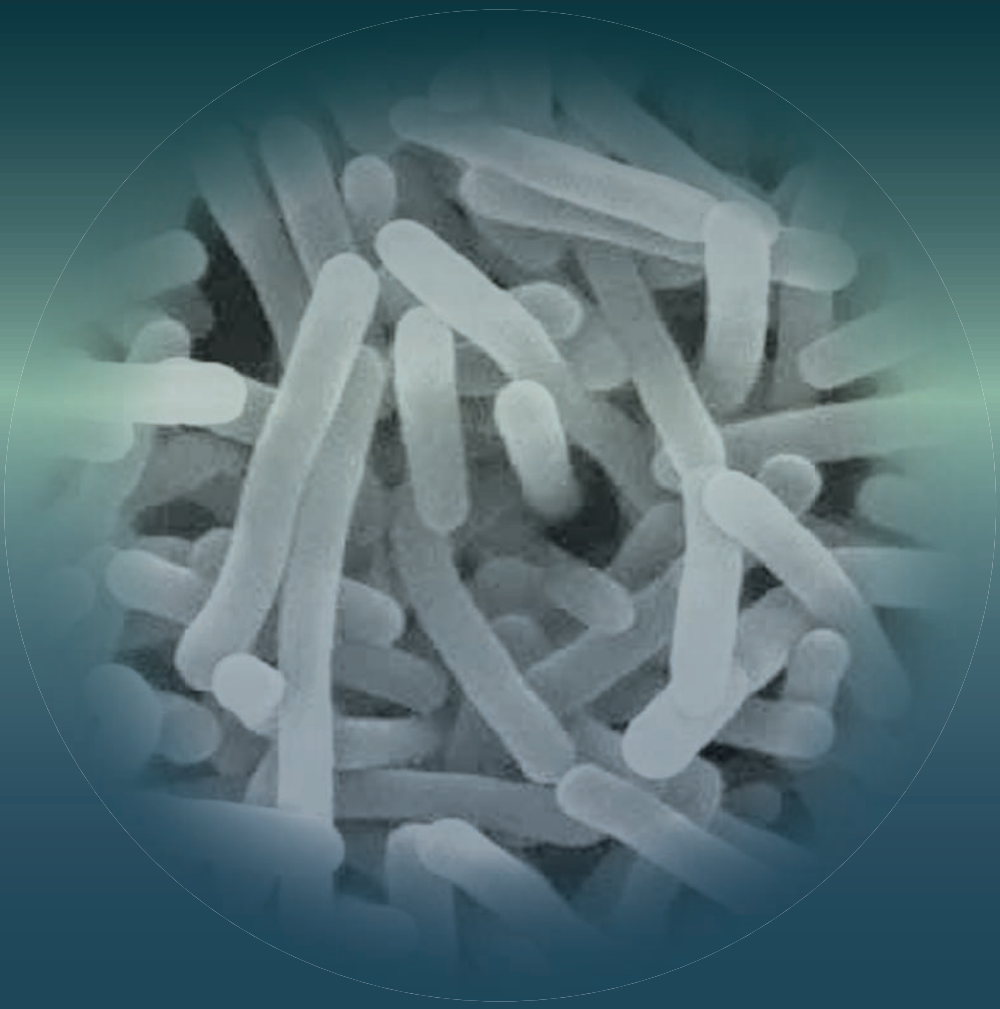
Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture



IAEA
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IRRADIATION TO ENSURE THE SAFETY AND QUALITY OF PREPARED MEALS

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IRRADIATION TO ENSURE THE SAFETY AND QUALITY OF PREPARED MEALS

RESULTS OF THE COORDINATED RESEARCH PROJECT
ORGANIZED BY THE JOINT FAO/IAEA DIVISION OF
NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE (2002–2006)

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FOREWORD

The consumption of prepared meals has increased enormously during the last decade, not only in developed countries but also in developing countries where many types of ethnic foods are now also prepared as convenience foods. Consumer studies carried out on the consumption of these types of foods have shown that perceived time pressures contribute positively to the purchase of both prepared meals and takeaway meals. Other reasons are also mentioned in the literature, among them are the increasing proportion of working women outside of the home, not enjoying cooking for oneself and the need of family members to eat at different times.

Traditionally, prepared meals are retort processed, or, more recently, stored frozen, whereas an increasing demand exists for chilled commodities, partly due to their fresh appearance. However, the chilled prepared meals are non-sterile and potential survival of some pathogenic microorganisms and/or post-processing contamination before packaging creates microbiological risks and a considerable limitation of shelf life. This is particularly important for countries where the microbiological safety of many ethnic prepared meals is questionable and their shelf life limited due to the conditions under which they are produced, stored and distributed.

Food irradiation used on its own, or in combination with other technologies, could significantly enhance the microbial safety of such products as well as extend their shelf life. Although extensive research has been carried out on the microbiological, chemical, nutritional and sensorial effects of irradiating individual uncooked food items, little work has been reported on the irradiation of complex food systems such as prepared meals.

In 2002, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture initiated in a Coordinated Research Project on Irradiation to Ensure the Safety and Quality of Prepared Meals. This project included the studies of participants from different regions of the world. In total, more than 50 different prepared meals were investigated. This publication presents the results of studies conducted over a five year period (2002–2006) on the safety, shelf life and overall quality of the meals stored under ambient, chilled or frozen conditions.

Special thanks are due to J. Farkas and C. Mohácsi-Farkas who assisted in finalizing this manuscript for publication. The IAEA officer responsible for selecting the participants and organizing the first research coordination meeting in Vienna was P. Loaharanu. The officer responsible for subsequent follow-up of the project, as well as the preparation of this publication, was T. Rubio-Cabello of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

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SUMMARY

1. INTRODUCTION

The prepared convenience foods sector has become a significant part of the economy of many developed countries, with a similar trend evolving in developing countries, where many types of ethnic foods are now also prepared as convenience foods. For example, the prepared convenience foods sector in Ireland is a significant part of the Irish economy. In 2001, just under half of the sector's total output was exported for a value of €841 million, representing a 12% annual increase. The sector's strong growth both in exports and in total sales has made it one of the fastest growing sectors of the food industry in many countries.

Consumer studies carried out on convenience foods have shown that perceived time pressures contribute positively to the purchase of both prepared and takeaway meals. Other reasons found to contribute positively to the purchase of prepared meals include the convenience of not cooking for oneself, a value for money perception of convenience foods and different eating times of family members. With rapid urbanization and change in socioeconomic status, and an ever increasing proportion of working women, the tendencies are similar in developing countries.

Traditionally, prepared meals are retort processed or, more recently, stored frozen, whereas an increasing demand exists for chilled commodities, partly due to their fresh appearance, which is more appealing to the consumer than canned or frozen meals. Freezing and retort processing are also more energy demanding. Chilled prepared foods, however, are non-sterile and potential survival of some pathogenic microorganisms and/or post-processing contamination before packaging create microbiological risks and experience a considerable shortening of shelf life. For example, in Germany, a survey of prepared cooked meat products showed an incidence rate of 3.7% for *Listeria monocytogenes*. In the ethnic Korean food, kimbab, *Salmonella* spp.[1] is found in some instances, especially in the summer[2].

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[1] NOACK, D.J., JOECKEL, J., *Listeria monocytogenes*: Occurrence and significance in meat and meat products and experience with recommendation for its detection and assessment, Fleischwirtschaft **73** (1993) 581–584.

[2] KANG, Y.S., et al., Prevalence of *Staphylococcus aureus* in kimbab, J. Food Hygiene Safety **19** (2002) 35–44.

Even frozen foods are not necessarily safe if already contaminated by pathogenic bacteria. This safety problem is even more aggravated in the case of chilled foods, which are more vulnerable to temperature abuse or instabilities in the 'cold chain'. This can result in the growth of psychrotrophic pathogens. In addition to this problem, chilled prepared meals have a limited shelf life under chilling conditions, thereby limiting the geographical area in which they can be marketed. Therefore, technologies that will improve their microbiological safety, while extending the shelf life, are required.

As a consequence of the increased national and international interest in the marketing of convenience and prepared foods, the food industry needs to find new ways of producing safe and high quality prepared meals. One technology with a particular potential to achieve these objectives is food irradiation.

It is thought that research into the application of ionizing radiation to products such as prepared meals could be of ultimate benefit to consumers, industry and trade. This is particularly important for countries where the microbiological safety of many ethnic prepared meals is questionable and their shelf life limited due to the conditions under which they are produced, stored and distributed. Food irradiation used on its own or in combination with other technologies could significantly enhance the microbial safety of such products as well as extending shelf life. This is of special importance for the most vulnerable individuals in society, such as the immunocompromised. It is estimated that this group comprises 20% of the total world population, whether they are hospitalized or not.

Although extensive research has been carried out on the microbiological and sensorial effects of irradiating individual uncooked food items, little work has been reported on the irradiation of complex food systems such as prepared meals. In this coordinated research project (CRP), the potential of using irradiation technology for convenience foods has been investigated with regard to safety, shelf life and overall quality, particularly in terms of sensory acceptance. A wide range of ethnic meals, as well as meal components, were investigated with the objective of meeting continual changes in consumer demand worldwide.

Other aspects of the CRP included the adoption of a hazard analysis critical control point (HACCP) system for prepared meals and research into consumer willingness to purchase irradiated food at a premium price. The scope of the CRP was therefore wide ranging.

The overall objective of this CRP was to evaluate the effectiveness of irradiation as a method of ensuring the microbiological safety and extending the shelf life of prepared meals, stored under ambient, chilled or frozen conditions, and to evaluate the sensory quality of the treated products.

The specific objective of the CRP was to use validated procedures for irradiation treatment and process control, and to use validated methods for assessing microbiological safety and quality as well as the sensory quality of prepared meals, mainly of ethnic origin.

2. ACHIEVEMENTS

2.1. General achievements

The participants carried out research into more than 50 different prepared meals. Table 1 summarizes the dishes investigated as well their composition, intrinsic qualities and the analyses carried out in order to determine their overall safety and quality.

2.1.1. Animal based prepared meals

The efficacy of radiation processing for the microbiological safety and quality of more than 30 prepared meals with beef, chicken, pork, mutton or prawns as a major component was investigated (Table 1). The meals included cannelloni, empanadas, sandwiches (ham and cheese), chicken pie, soups, Yunan chicken, Thai spicy chicken, poached chicken, chicken chilli, chicken masala, bulgogi, galbi, kubba, borak, sheesh tawoq, prawn pulao and khichadi. The optimum gamma radiation doses were found to be in the range of 2–4 kGy for a majority of the meals in order to achieve microbiological safety and the desired sensory quality. Challenge studies with pathogens such as *Escherichia coli*, *L. monocytogenes*, *Staphylococcus aureus* and *Salmonella* spp., revealed that doses employed eliminated the test organisms, thus demonstrating improvement in the microbiological safety of these products. In general, the shelf life of the meals was extended from one week to more than three weeks at chilled temperatures, depending upon the characteristics of the meals. No significant changes were observed with regard to physical and chemical properties such as pH, water activity and lipid peroxidation of the meals. There was no significant difference in the overall acceptability of the meals at the optimal doses of gamma radiation. In addition, it was found that using natural antioxidants such as vitamin C, vitamin E and flavonoids (quercetin, epicatechin and resveratrol) could enhance the quality of the meat component of irradiated meals by successfully reducing the occurrence of oxidative rancidity. Shelf life studies conducted at abuse temperatures emphasized the importance of maintaining the ‘cold chain’ during production and storage of irradiated prepared meals.

TABLE 1. PREPARED MEALS/MEAL COMPONENTS STUDIED

Country	Animal based products	Vegetable/fruit based products/miscellaneous
Argentina	Cannelloni Empanadas Sandwich (ham and cheese) Chicken pie Eggs (hard boiled)	Salad Fruit salad Custard Bread pudding Fruit based dessert
Ghana	Poached chicken meal Beef tripe (with jollof rice) Fried fish (with waakye)	Jollof rice (rice cooked in tomato sauce) Waakye (co-boiled rice and cowpeas, and vegetable salad)
Hungary	Cordon bleu (reconstituted turkey meat with cheese and ham) Filled pasta products (tortellini)	
India	Prawn masala Prawn pulao Mutton shami kebabs Chicken chilli Chicken biryani Prawn pulao	Poha (made from rice pressed into flakes) Upma (cooked semolina/ cracked wheat and spices) Mixed vegetables Rice Vegetable pulao Khichadi (cereal/legume gruel)
Indonesia	Black soup Oxtail soup Chicken vegetable soup Chicken sweetcorn soup Yunan chicken	Croquette Risolle Spring rolls
Republic of Korea	Bulgogi (cooked beef with vegetables) Galbi (marinated beef ribs)	Kimbab (cooked rice rolled in dried seaweed) Kimchijumeokbab (cooked rice mixed with fermented vegetables and fried pork)
South Africa	Beef biltong Ready-to-eat bovine tripe	
Syrian Arab Republic	Kubba (spicy mince coated with ground wheat) Borak (lamb in dough/cheese in dough) Sheesh tawoq (spicy, boneless chicken)	

TABLE 1. PREPARED MEALS/MEAL COMPONENTS STUDIED (cont.)

Country	Animal based products	Vegetable/fruit based products/miscellaneous
Thailand	Thai spicy chicken basil rice (kao ka pao kai) Stir fried rice noodle with dried shrimp (pad Thai) Steamed sticky rice with roasted chicken and papaya salad (kao neaw som tom)	
United Kingdom	Chicken masala Minced beef patties Salmon meat patties	

2.1.2. Vegetable–fruit based prepared meals and miscellaneous meals

Radiation processing of two of the most popular vegetarian meals consumed in India, namely vegetable pulao and mixed vegetables, was standardized. These samples were found to be contaminated by potentially pathogenic bacteria such as *S. aureus* and spoiled within two weeks. Contrary to this, no viable bacterial growth was observed in samples treated with gamma radiation (2 kGy) up to 30 d in storage. It was concluded that these meals treated with 2 kGy were microbiologically safe, with a shelf life of a month. This would be a significant advantage to processors, retailers and consumers.

Studies carried out showed that some of the vegetable based preparations, including fruit salad, custard and bread pudding, could be decontaminated by radiation processing for immunocompromised patients. A dessert composed of fresh apples and pear cubes mixed with strawberry flavoured gelatine jelly and soft white cheese, packaged in polypropylene and refrigerated at 5°C, was successfully decontaminated by 1.5 kGy of gamma radiation, attaining a 3 log cycle reduction in total bacteria counts (TBCs) with acceptable sensory quality throughout a week of storage, which doubled its shelf life. A *Salmonella enteritidis* challenge test showed that this dose was sufficient to reduce its counts by 6 log cycles, which ensured a good level of safety.

Gamma radiation of a carrot, hard boiled egg and tomato salad at a dose of 2 kGy, packaged in polypropylene, covered with PVC film and stored at 5°C was sufficient to attain a 6 log cycle reduction in *S. enteritidis* counts. The TBCs were reduced by 3 or 4 log cycles with few detrimental effects on sensory quality.

The sensory quality of cooked rice irradiated at more than 2 kGy was found to be unacceptable in terms of texture and colour. For steamed sticky rice, a dose of 3 kGy was enough to control *L. monocytogenes* and *E. coli* during chilled storage when stored for more than 8 weeks. Doses of 4 kGy are recommended for stir-fried rice noodles with sauces and dry shrimp. Similar results were obtained for jollof rice, 3 kGy extended its shelf life under chilled storage for 28 d without significant effects on its sensory quality.

Gamma irradiation with doses of 5–7 kGy of four frozen soups which were vacuum packaged within laminated pouches of polyester/aluminium foil/LLDPE and made of different basic materials, having moisture contents between 69 and 86%, could reduce microbial load by 2 or 3 log cycles and extend the shelf life to 3 months at 5 ± 2°C, without impairing sensory quality. A challenge test result indicated that 5–7 kGy doses were sufficient to reduce the population of *Clostridium sporogenes* by 6 or 7 log cycles.

2.2. Additional achievements

In addition to the general achievements, some participants carried out studies on the following issues:

- Predictive microbiological modelling;
- HACCP;
- Consumer studies;
- Studies with immunocompromised patients.

2.2.1. Predictive microbiological modelling

As a result of intensive predictive microbiological modelling activities, several computer programs and software became available recently for facilitating microbiological risk assessment. Among these tools, the establishment of ComBase, an international database and its predictive modelling softwares of the Pathogen Modelling Program (PMP) set up by the USDA Eastern Regional Research Center and the Food Micromodel/Growth Predictor by the United Kingdom's Institute of Food Research have been the most important. Under the CRP, the PMP 6.0 software version of ComBase was used for a preliminary trial to compare observed growth of selected test organisms in relation to irradiated food studied under previous FAO/IAEA coordinated food irradiation research projects (D6.10.23 and D6.20.07). The results of challenge tests with *L. monocytogenes* inoculum in untreated or irradiated experimental batches of semi-prepared breaded turkey meat steaks (cordon bleu), sliced tomatoes, sliced watermelon, sliced cantaloupe and sous vide

processed mixed vegetables, as well as *S. aureus* inoculum of a pasta product, tortellini, were compared with their respective growth models under relevant environmental conditions. This comparison showed good fits in the case of non-irradiated and high moisture food samples, whereas growth of radiation survivors lagged behind the predicted values. Further progress in this CRP and increasing efforts to invest in the automation of microbiological measurements and the development of systematically organized databases for the collected data would be of great interest.

2.2.2. HACCP

The objective of the work was to introduce a modified HACCP based analysis for irradiated prepared meals that addresses potential safety hazards, as well as sensorial failures, and economic risks, while pin-pointing failure modes specific to the radiation pasteurization aspects. The analysis covered all production inputs and stages, and all foreseen failure modes related to the physical, chemical and biological hazards of the prepared meals: raw materials, packaging and prepared meals. A practical 10 step approach to implement the suggested modified HACCP plan, from comprehensive analysis to validated protocol, was further provided. At the final stage of this study, a collaborative HACCP was carried out on the ethnic foods of Indonesia as an example of these specific types of food. Approaching the industrial stage of safe prepared meals was further assessed in this study. In view of the currently accelerating consumer demand and industrial production of prepared meals and convenience foods, the multistage approach to ingredient safety seems preferred from safety, sensory, irradiation and economic perspectives. With proper orchestration of the radiation stages within the production chain, it would achieve better food quality and safety and at the same time use less radiation, in two or more stages.

2.2.3. Consumer studies

The objective of the consumer studies was to assess and evaluate consumers' perceptions, acceptance and willingness to pay for irradiated ground beef patties. Determining consumers' willingness to pay a premium for irradiated food products is important because this is a major factor that would determine the potential marketability and success of the product. In addition, food irradiation adds to the costs of production and these costs must be capable of being covered by the price premium before any food manufacturer or retailer will consider selling the irradiated product. Studies carried out using both survey and experimental economics methodologies generally suggest that

information about the nature of food irradiation technology increases consumer acceptance of irradiated prepared and processed ground beef. The research findings also indicated that consumers are willing to pay a premium for irradiated ground beef. Most of the participants also conducted consumer sensory evaluation tests to establish acceptability of these irradiated prepared meals.

2.2.4. Study with immonocompromised patients

Regarding the experience with immunocompromised patients, it was concluded that ionizing radiation, in combination with good manufacturing practices and refrigeration, improve their feeding quality as more diversification is allowed, with both nutritional and psychological benefits. The level of microbial decontamination attained would afford offering highly desired unusual meals to these persons, without risk of foodborne diseases. Besides, nutritionists were satisfied with the results obtained because the shelf life extension due to irradiation would allow, in the future, a reasonable flow of meals provided to a hospital by a catering service, for instance, once a week, without the food losing its fresh nutritive condition in consequence of overcooking or freezing.

This was the first approach carried out in Argentina between immunocompromised patients and food irradiation. Much more work should be undertaken to widen meal variety and to publicize this method to other patients, health institutes, catering services and supermarkets.

3. SUMMARY OF RESULTS

A summary of the products studied, their composition, intrinsic qualities and the results of analyses are given in Table 2, while an overall summary of the effect of irradiation on the safety, shelf life and quality of the products listed in Tables 1 and 2 is presented in Table 3.

TABLE 2. SUMMARY OF SAFETY AND QUALITY PARAMETERS STUDIED

Country	Prepared meal(s) studied	Composition of prepared meal(s)	Intrinsic parameters	Safety and quality parameters		
				Microbiological	Sensorial	Chemical/physical
Argentina*	Cannelloni in tomato sauce	Wheat dough wrapping (raw) Filling: cooked spinach, veal meat, cheese	pH, water activity	*L. innocua*	Overall acceptability using a 9 point hedonic scale	
	Salad	Grated carrot Whole cherry tomatoes, hard boiled egg		*S. enteritidis*		
	Empanadas	Wheat dough wrapping (raw) Filling: boiled chicken breast, vegetables		*S. enteritidis*		
	Fruit salad in gelatine with white cheese	Fresh apples, pears, commercial strawberry gelatine, soft cheese		*S. enteritidis*		
	Sandwiches	Wheat bread, butter, mayonaise, ham and cheese		*L. monocytogenes*		
	Pies	Wheat dough wrapping, chicken and vegetable fillings		*S. typhimurium*		
	Custard			*S. typhimurium*		
	Bread pudding			*L. monocytogenes,* *S. typhimurium*		

TABLE 2. SUMMARY OF SAFETY AND QUALITY PARAMETERS STUDIED (cont.)

Country	Prepared meal(s) studied	Composition of prepared meal(s)	Intrinsic parameters	Safety and quality parameters		
				Microbiological	Sensorial	Chemical/physical
Ghana	Poached chicken meat	Chicken, rice, carrots	pH, water activity	Total viable count, *E. coli, S. aureus*	Triangle test, overall acceptability using a 9 point hedonic scale	FFA
	Jollof rice	Rice cooked in tomato sauce	As above	Total viable count, *Salmonella* spp., *S. aureus, E. coli*, yeasts and moulds, *Shigella*	Descriptive sensory analyses	FFA Peroxide value
	Waakye	Co-boiled rice and cowpeas	As above	TBC, total coliforms, yeasts and moulds		
	HACCP prepared ready-to-eat meals	14 different commercially available airline meals		TBC, total coliforms, *Salmonella/ Shigella*, yeasts and moulds		
Hungary	Tortellini	Pasta filled with meat/vegetables	pH, water activity	TBC, *S. aureus*	Hedonic scores, ranking tests	Thiamine
	Cordon bleu	Pre-fried, irradiated slices/steaks of reconstituted turkey meat filled with slices of ham and cheese	As above	TBC, lactic acid bacteria, *L. monocytogenes,* Clostridia	As above	TBARS
	Mechanically deboned turkey meat			TBC, enterobacteria count		TBARS Cholesterol oxidation products

TABLE 2. SUMMARY OF SAFETY AND QUALITY PARAMETERS STUDIED (cont.)

Country	Prepared meal(s) studied	Composition of prepared meal(s)	Intrinsic parameters	Safety and quality parameters		
				Microbiological	Sensorial	Chemical/physical
India	Prawn masala Prawn pulao Mutton shami kebabs Chicken chilli Chicken biryani Prawn pulao Khichadi (cereal/ legume gruel) Poha Upma Mixed vegetables Rice Vegetable pulao	Vegetables, chicken, rice, prawns, garlic, ginger paste	pH, water activity	TBC, *S. aureus*, *B. cereus*, *L. monocytogenes,* faecal coliforms, yeasts and moulds, aerobic spore count	Overall acceptability using a 10 point hedonic scale	TBARS
Indonesia	Black soup	Beef, *Pangium edule*, shallot, garlic, roasted coriander, red chilli, ginger, lemon leaf, roasted fish paste, turmeric, ginger root, lemon grass, *Kaempferia galanga*, salt, palm sugar, bay leaf, palm oil, water	pH, fat, carbohydrate, protein	TBC, yeasts and moulds, coliforms, *E. coli, Salmonella* spp., *S. aureus*, *C. perfringens*	Overall acceptability using a 5 point hedonic scale	
	Oxtail soup	Oxtail, shallot, garlic, salt, palm oil, water, onion, nutmeg, cloves, white pepper, onion leaf, celery, margarine				

TABLE 2. SUMMARY OF SAFETY AND QUALITY PARAMETERS STUDIED (cont.)

Country	Prepared meal(s) studied	Composition of prepared meal(s)	Intrinsic parameters	Safety and quality parameters		
				Microbiological	Sensorial	Chemical/physical
	Chicken and vegetable soup	Chicken, shallot, garlic, salt, water, nutmeg, white pepper, onion leaf, celery, margarine, sugar, carrot, green beans, broccoli, sugar peas				
	Chicken sweetcorn soup	Chicken, salt, water, nutmeg, sweetcorn, chicken sausage, carrot, egg, cornstarch				
	Spring rolls	Wheat based sheet filled with cooked shrimps and chicken as well as vegetables (including young bamboo shoots)	As above plus water activity			
	Risolle	Wheat based sheet filled with cooked chicken, vegetables	As above plus water activity			
	Croquette	Potato based sheet filled with beef and vegetables	As above plus water activity			
	Yunan chicken	Specially salted chicken, marinated in herbs and spices (cooked in a steamer)	As above plus water activity			Thiamine, peroxide value

TABLE 2. SUMMARY OF SAFETY AND QUALITY PARAMETERS STUDIED (cont.)

Country	Prepared meal(s) studied	Composition of prepared meal(s)	Intrinsic parameters	Safety and quality parameters		
				Microbiological	Sensorial	Chemical/physical
Israel	Black soup	Beef, football fruit (*P. edule)*, shallot, garlic, roasted coriander, red chilli, ginger, lemon leaf, roasted fish paste, turmeric, ginger root, lemon grass, spice (*K. galanga)*, salt, palm sugar, bay leaf, palm oil, water	HACCP parameters	HACCP parameters	HACCP parameters	HACCP parameters
	Oxtail soup	Oxtail, shallot, garlic, salt, palm oil, water, onion, nutmeg, cloves, white pepper, onion leaf, celery, margarine				
	Chicken and vegetable soup	Chicken, shallot, garlic, salt, water, nutmeg, white pepper, onion leaf, celery, margarine, sugar, carrot, green beans, broccoli, sugar peas				
	Chicken sweetcorn soup	Chicken, salt, water, nutmeg, sweetcorn, chicken sausage, carrot, egg, cornstarch				
	Spring rolls					
	Risolle					
	Croquette					

TABLE 2. SUMMARY OF SAFETY AND QUALITY PARAMETERS STUDIED (cont.)

Country	Prepared meal(s) studied	Composition of prepared meal(s)	Intrinsic parameters	Safety and quality parameters		
				Microbiological	Sensorial	Chemical/physical
Republic of Korea	Bulgogi Galbi Kimbab	Cooked rice rolled in dried laver (seaweed)	pH, water activity	Thermophilic bacteria, coliforms, *S. aureus, E. coli*, *S. typhimurium, B. cereus, Listeria ivanovii,* TBC	Overall acceptability using a 9 point hedonic scale	TBARS, DPPH, protease activity
	Kimchijumeokbab	Cooked rice mixed with fermented vegetables and fried pork		TBC		
South Africa	Biltong	Salted, dried, intermediate moisture meat product	Moisture, NaCl, water activity, fat, pH	*S. aureus*	Multiple difference testing Overall acceptability using a 9 point hedonic scale	TBARS
	Ready-to-eat bovine tripe	Beef tripe		*C. perfringens*	9 point hedonic scale	
Syrian Arab Republic	Kubba	Ground wheat, beef, spices, lamb, onion, fat, pistachio	Moisture, fat, ash, protein, water activity, pH	TBC, coliforms, yeasts and moulds, *Salmonella* spp., *E. coli*	Overall acceptability using a 5 point hedonic scale	Lipid oxidation, TVBN, total acidity Proximate analyses
	Borak	Dough, eggs, lamb, onion, spices				
	Cheese borak	Dough, eggs, cheese				
	Sheesh tawoq	Spicy, boneless chicken				

TABLE 2. SUMMARY OF SAFETY AND QUALITY PARAMETERS STUDIED (cont.)

Country	Prepared meal(s) studied	Composition of prepared meal(s)	Intrinsic parameters	Safety and quality parameters		
				Microbiological	Sensorial	Chemical/physical
Thailand	Kao ka pau kai	Cooked rice, chicken, vegetable oil, chilli, fish sauce, water, basil leaves	Moisture, pH	*L. monocytogenes*, *E. coli*, *S. typhimurium*	Overall acceptability using a 9 point hedonic scale	Instrumental texture analyses Laboratory colour measurements
	Pad Thai	Stir-fried rice noodle with dried shrimp		*L. monocytogenes*, *E. coli*		
	Kao neaw som tom	Sticky rice, roasted chicken and papaya salad		*L. monocytogenes*, *E. coli*		
United Kingdom	Chicken masala	Chicken, onion, tomato, water, yoghurt, coconut, red pepper, tomato puree, rapeseed, oil modified starch, coriander leaf, salt, ginger, cayenne pepper, malt extract, turmeric		TBC, *Pseudomonas* spp., psychrotrophos, lactic acid bacteria, coliforms		TBARS, vitamins B_1 and E, cyclobutanone (EN1786)
	Beef patties			TBC, *Pseudomonas* spp., psychrotrophs, anaerobic spores		TBARS CIELAB colour measurements Addition of natural antioxidants
	Salmon meat patties					CIELAB colour measurements Addition of natural antioxidants

TABLE 2. SUMMARY OF SAFETY AND QUALITY PARAMETERS STUDIED (cont.)

Country	Prepared meal(s) studied	Composition of prepared meal(s)	Intrinsic parameters	Safety and quality parameters		
				Microbiological	Sensorial	Chemical/physical
United States of America	Ground beef patties	Beef	Consumer demand and acceptance parameters		Consumer demand and acceptance parameters	

[*] Additional to TBC, mould and yeast counts, spore forms, anaerobes, *Salmonella* spp., *S. aureus,* coliforms.
POV: total volatile basic nitrogen, DPPH: 1,1-diphenyl-2-picrylhydrazyl, FFA: free fatty acids.

TABLE 3. SUMMARY OF THE EFFECTS OF IRRADIATION ON THE SAFETY, SHELF LIFE AND QUALITY OF PRODUCTS STUDIED

Country	Prepared meal(s) studied	Effective irradiation dose (kGy)	Packaging conditions	Temperature of storage (ºC)	Inoculated pathogens (surrogates)	Improvement in safety/shelf life
Argentina	Cannelloni in tomato sauce	3	Aerobic	5 ± 2	*L. innocua* (*S. enteritidis*)	The irradiation treatment rendered a microbiologically safe and sensorially acceptable prepared meals studied at least 8 storage days at refrigeration temperatures (shelf life extended at least threefold).
	Salad	2	Aerobic	5 ± 2	*L. innocua* (*S. enteritidis*)	
	Empanadas	3	Aerobic	5 ± 2	*L. innocua* (*S. enteritidis*)	

TABLE 3. SUMMARY OF THE EFFECTS OF IRRADIATION ON THE SAFETY, SHELF LIFE AND QUALITY OF PRODUCTS STUDIED (cont.)

Country	Prepared meal(s) studied	Effective irradiation dose (kGy)	Packaging conditions	Temperature of storage (°C)	Inoculated pathogens (surrogates)	Improvement in safety/shelf life
	Fruit salad in gelatine with white cheese	1.5	Aerobic	5 ± 2	(*S. enteritidis*)	
	Sandwiches	2.5	Aerobic	5 ± 2	*L. monocytogenes* ATCC 15313	
	Pies	3.5	Aerobic	5 ± 2	*L. monocytogenes* ATCC 15313	
	Custard	3	Aerobic	5 ± 2	*(S. typhimurium*)	
	Bread pudding	4	Aerobic	5 ± 2	*L. monocytogenes* ATCC 15313 (*S. enteritidis* ATCC 13076)	
Ghana	Poached chicken	3	Aerobic	3–5	*E. coli* (D10 = 0.19 kGy) *S. aureus* (D10 = 0.27 kGy)	Eliminated test pathogens From 7 d (control) to 21 d at 3–5°C
	Jollof rice with beef tripe and sauce	3		3–5	*E. coli* (D10 = 0.17 kGy) *S. aureus* (D10 = 0.26 kGy) *Salmonella* spp. (D10 = 0.29 kGy)	Eliminated test pathogens From 7 d (control) to 21 d at 3–5°C

TABLE 3. SUMMARY OF THE EFFECTS OF IRRADIATION ON THE SAFETY, SHELF LIFE AND QUALITY OF PRODUCTS STUDIED (cont.)

Country	Prepared meal(s) studied	Effective irradiation dose (kGy)	Packaging conditions	Temperature of storage (°C)	Inoculated pathogens (surrogates)	Improvement in safety/shelf life
	Waakye with fried fish, vegetable salad and tomato sauce	3		27–32	*E. coli* (D10 = 0.27 kGy) *S. aureus* (D10 = 0.33 kGy) *Salmonella* spp. (D10 = 0.44 kGy)	Shelf life studies not conducted
Hungary	Cordon bleu (water activity = 0.96, pH = 6.2–6.3)	2	MAP (20% CO_2 + 80% N_2)	5–9	*L. monocytogenes* 4ab No.10 (D10 ~ 0.80 kGy)	From 2 weeks (control) to 4 weeks at 5°C
	Tortellini (water activity = 0.96, pH = 5.5–6.1)	3	MAP (20% CO_2 + 80% N_2)	15	*S. aureus* ATCC 6538 (D10 < 0.80 kGy)	From 1 week (control) to 2 weeks at 9°C Less than 2 weeks (control) to 4 weeks at 15°C
India	Prawn masala	3	Aerobic	0–3		Pathogens eliminated (*L. monocytogenes, S. aureus, E. coli, Salmonella*)
	Chicken chilli				*S. aureus* ATCC 6538P (D10 = 0.37 kGy) *B. cereus* MTCC 470 (D10 = 0.47 kGy)	Shelf life extended to at least 2 weeks at 0–3°C
	Mutton shami kebabs				*S. aureus* ATCC 6538P (D10 = 0.37 kGy) *B. cereus* MTCC 470 (D10 = 0.47 kGy)	

TABLE 3. SUMMARY OF THE EFFECTS OF IRRADIATION ON THE SAFETY, SHELF LIFE AND QUALITY OF PRODUCTS STUDIED (cont.)

Country	Prepared meal(s) studied	Effective irradiation dose (kGy)	Packaging conditions	Temperature of storage (ºC)	Inoculated pathogens (surrogates)	Improvement in safety/shelf life
	Prawn pulao					
	Chicken biryani					
	Khichadi (cereal/ legume gruel)	2	Aerobic	0–3		Pathogens eliminated (*L. monocytogenes*, *S. aureus, E. coli*, *Salmonella*) Shelf life extended to at least 4 weeks at 0–3ºC
	Poha					
	Upma					
	Mixed vegetables					
	Rice					
	Vegetable pulao					
Indonesia	Black soup	5–7 (irradiated under cryogenic conditions)	Vacuum packaged in laminated pouch (anaerobic)	5 ± 2		Pathogens eliminated (*E coli, Staphylococcus* spp., *C. perfringens*) Irradiation extended the shelf life up to 3 months at 5 ± 2ºC
	Oxtail soup					
	Chicken and vegetable soup					
	Chicken sweetcorn soup					

TABLE 3. SUMMARY OF THE EFFECTS OF IRRADIATION ON THE SAFETY, SHELF LIFE AND QUALITY OF PRODUCTS STUDIED (cont.)

Country	Prepared meal(s) studied	Effective irradiation dose (kGy)	Packaging conditions	Temperature of storage (ºC)	Inoculated pathogens (surrogates)	Improvement in safety/shelf life
	Spring rolls	7				Pathogens eliminated (*E. coli, Staphylococcus* spp., *C. perfringens*) Microbiological shelf life: 1 month at 5 ± 2ºC Sensory quality not acceptable after irradiation due to textural defect in young bamboo shoot
	Risolle	3–7				Pathogens eliminated (*E. coli, Staphylococcus* spp., *C. perfringens*) Irradiation extended the shelf life up to 3 months at 5 ± 2ºC
	Croquette	5–7				Pathogens eliminated (*E coli, Staphylococcus* spp., *C. perfringens*) Irradiation extended the shelf life up to 3 months at 5 ± 2ºC
	Yunan chicken	3–5			(*S. typhimurium*) (D10 = 0.28 kGy) *Pseudomonas aeruginosa* (D10 = 0.17 kGy) *E. coli* 0157 (D10 = 0.12 kGy) *Campylobacter* spp. (D10 = 0.09 kGy) *L. monocytogenes* (D10 = 0.66 kGy)	Test pathogens eliminated by irradiation From 6 weeks (control) to 9 weeks (3 and 5 kGy) at 5 ± 2ºC

TABLE 3. SUMMARY OF THE EFFECTS OF IRRADIATION ON THE SAFETY, SHELF LIFE AND QUALITY OF PRODUCTS STUDIED (cont.)

Country	Prepared meal(s) studied	Effective irradiation dose (kGy)	Packaging conditions	Temperature of storage (°C)	Inoculated pathogens (surrogates)	Improvement in safety/shelf life
Republic of Korea	Bulgogi	2.5	Aerobic	0–5		Eliminated *B. cereus*, coliforms From 2 weeks (control) to 4 weeks (2.5 kGy)
	Galbi	2.5–5	Aerobic (but vacuum recommended)	0–5	*B. cereus* KCTC 1012 (D10 = 0.66 kGy) *S. aureus* KCTC 1916 (D10 = 0.59 kGy) *E. coli* KCTC 1682 (D10 = 0.54 kGy) (*S. typhimurium*) KCTC (D10 = 0.63 kGy)	Eliminated all inoculated pathogens From 2 weeks (control) to 4 weeks (2.5 kGy) at 0–5°C
	Kimbab	1	Aerobic	10	*S. aureus* KCTC 1916 (D10 = 0.31 kGy) *E. coli* KCTC 1682 (D10 = 0.42 kGy) (*S. typhimurium*) KCTC (D10 = 0.44 kGy) *L. ivanovii* KCTC 3444 (D10 = 0.43 kGy)	Eliminated all tested pathogens From 12 h (control) to 36 h (1.0 kGy) at 10°C
	Kimchijumeokbab	3	Aerobic	10		From 12 h (control) to 36 h (3 kGy) at 10°C

TABLE 3. SUMMARY OF THE EFFECTS OF IRRADIATION ON THE SAFETY, SHELF LIFE AND QUALITY OF PRODUCTS STUDIED (cont.)

Country	Prepared meal(s) studied	Effective irradiation dose (kGy)	Packaging conditions	Temperature of storage (°C)	Inoculated pathogens (surrogates)	Improvement in safety/shelf life
South Africa	Biltong (water activity = 0.979, pH = 5.32)	4	Vacuum	Ambient	*S. aureus* ATCC 9441	Eliminated test pathogen
	Ready-to-eat bovine tripe	9	Aerobic (boil after packaging)	5 15		No *C. perfringens* after irradiation but survival of heat and irradiation resistant aerobic spore formers Extends shelf life to at least 2 weeks at both 5 and 15°C Control sample spoils after 3 days at 15°C Elimination of test pathogen
		9	Anaerobic (boil before vacuum packaging)	5 15	*C. perfringens* ATCC 13124	Shelf life of irradiated samples at least 7 d at both 5 and 15°C Control sample spoils after 4 d at 15°C
Syrian Arab Republic	Kubba	4–6	Aerobic	0–4		From less than 1 week (control) to 3 weeks at 4 or 6 kGy
	Borak	4–6	Aerobic	0–4	*Salmonella* spp. (D10 = 0.47 kGy) *E. coli* (D10 = 0.51 kGy)	Elimination of pathogens From less than 1 week (control) to 3 weeks at 4 kGy and at least 5 weeks at 6 kGy

TABLE 3. SUMMARY OF THE EFFECTS OF IRRADIATION ON THE SAFETY, SHELF LIFE AND QUALITY OF PRODUCTS STUDIED (cont.)

Country	Prepared meal(s) studied	Effective irradiation dose (kGy)	Packaging conditions	Temperature of storage (ºC)	Inoculated pathogens (surrogates)	Improvement in safety/shelf life
	Cheese borak	4–6	Aerobic	0–4	*Salmonella* spp. (D10 = 0.30 kGy) *E. coli* (D10 = 0.50 kGy)	Elimination of pathogens From less than 1 week (control) to 2 weeks at 4 kGy to at least 6 weeks at 6 kGy
	Sheesh tawoq	4–6	Aerobic	0–4		From 12 weeks (control) to at least 20 weeks (4 and 6 kGy)
Thailand	Kao ka pau kai – cooked rice	2	Aerobic	5	*L. monocytogenes* *E. coli*	Cooked rice: from 2 weeks (control) to at least 4 weeks at 5ºC
	– cooked chicken	2			*L. monocytogenes* *S. typhimurium*	Cooked rice: from 2 weeks (control) to atleast 4 weeks at 5ºC
	– basil leaves	1				Up to 2 weeks at 5ºC after irradiation
	Pad Thai – cooked rice noodle	4			*L. monocytogenes* (D10 = 0.29 kGy) *E. coli* (D10 = 0.69 kGy)	From 2 weeks (control) to at least 4 weeks
	– sauce and dry shrimp	3			*L. monocytogenes* (D10 = 0.49 kGy) *E. coli* (D10 = 0.68 kGy)	

TABLE 3. SUMMARY OF THE EFFECTS OF IRRADIATION ON THE SAFETY, SHELF LIFE AND QUALITY OF PRODUCTS STUDIED (cont.)

Country	Prepared meal(s) studied	Effective irradiation dose (kGy)	Packaging conditions	Temperature of storage (°C)	Inoculated pathogens (surrogates)	Improvement in safety/shelf life
	Kao neaw som tom – sticky rice	4			*L. monocytogenes* (D10 = 0.33 kGy) *E. coli* (D10 = 0.15 kGy)	
	– roasted chicken				*L. monocytogenes* (D10 = 0.51 kGy) *E. coli* (D10 = 0.52 kGy)	
	– papaya salad				*L. monocytogenes* (D10 = 0.23 kGy) *E. coli* (D10 = 0.44 kGy)	From 2 weeks (control) to at least 8 weeks
UK	Chicken masala	2–3	Aerobic	3		Shelf life of 14 d achieved with no adverse effect on vitamins B1 or E. Irradiation easily detectable using 2-alkylcyclobutanone method (EN1786)

TABLE 3. SUMMARY OF THE EFFECTS OF IRRADIATION ON THE SAFETY, SHELF LIFE AND QUALITY OF PRODUCTS STUDIED (cont.)

Country	Prepared meal(s) studied	Effective irradiation dose (kGy)	Packaging conditions	Temperature of storage (ºC)	Inoculated pathogens (surrogates)	Improvement in safety/shelf life
	Beef patties	2.5	Vacuum packed in polyethylene bags			During the 21 d post-irradiation, total viable counts were reduced/maintained at low levels in vacuum packs. Oxidative rancidity maintained at acceptable levels for sensory quality although appearance is poor until patties allowed to 'bloom up' in an oxygen atmosphere prior to use.
		2.5	Overwrapped with cling film	3		Total viable counts reduced to below limit of detection by irradiation over 21 d storage period. Oxidative rancidity increased with storage but maintained at acceptable levels for sensory quality by 2.5 kGy up to 21 d. Addition of the flavonoid antioxidants quercetin, epicatechin and reveratrol can reduce oxidative rancidity throughout shelf life.
	Salmon meat patties	2.5	Overwrapped	3		Addition of the flavonoid antioxidants quercetin, epicatechin and reveratrol can reduce oxidative rancidity throughout shelf life.

4. CONCLUSIONS

The CRP demonstrated that radiation processing of prepared meals results in safer food by eliminating pathogens and extending the shelf life by decreasing the number of spoilage organisms without significantly jeopardizing the overall quality. However, the work also highlighted the complexity and technological challenges of using radiation processing for multicomponent food systems such as prepared meals.

The safety of radiation processed products was demonstrated using challenge tests/inoculated pack studies using various pathogenic test organisms or their surrogates. Such products could satisfy or fulfil several niche markets. However, the proper storage temperature and maintenance of the cold chain are crucial factors for food safety and stability. Oxidative changes are sometimes enhanced by radiation treatment but it has been demonstrated that such changes can be counteracted by employing proper packaging conditions and using efficient antioxidant additives.

The implementation of a HACCP substantially reduced microbial counts, although some potential pathogens survived. Although the HACCP plan requires that the ready meals were held at 0 to –5ºC in order to suppress growth of survivors, the latter could proliferate during temperature abuse when power outages occur.

Strict hygiene practices during the manufacture of prepared meals are a prerequisite for the successful application of irradiation in order to ensure product safety, quality and extended shelf life.

In view of the insight gained about the quality changes that occur in the irradiated foods investigated under this CRP and the emerging needs of consumers (e.g. functional qualities of foods in relation to nutrition and health) further research activities are necessary.

In view of the increasing trend in consumer demand for safe prepared foods, the importance in the use of radiation pasteurization is likely to increase in the future. This was confirmed by the consumer studies carried out in the USA, the results of which were reported during the course of this CRP. Provision of information about the nature of food irradiation increases consumer acceptance and willingness to pay a premium for enhanced product safety and quality.

As a result of extensive predictive microbiological modelling activities during the last two decades, several computer programs and software became available recently for facilitating microbiological risk assessment. After validation of their satisfactory predictability, such softwares would be worthwhile to use because they offer efficient tools for risk estimation for

selected marketing scenarios and microbiological ecological parameters/stress factors in foods, thereby decreasing the necessity for very costly challenge tests.

This technology could potentially be advantageous for consumers, food manufacturers and traders worldwide as the foods are safer, have extended shelf lives and are high quality. The use of this technology would also make it possible to give a safer and wider variety of meals to specific target groups such as immunocompromised patients, as was shown by one of the participants.

Radiation treatment thereby offers the opportunity for a wider utilization and marketing of such high quality meals, including many ethnic food products.

5. RECOMMENDATIONS

When using any new food processing technology for complex food systems such as prepared meals, it is important to consider the requirements in terms of both product and process parameters to obtain the desired safety and quality effects. The oversimplified attitude of employing ionizing radiation as a technology to pasteurize an existing industrial meal product is often inadequate to meet the goals of extended shelf life and improved safety. The meal should be modified, first to extend its sensorial shelf life (e.g. texture) regardless of bacterial growth, and then pasteurized by irradiation. In particular, shelf life changes relating to conversion of bound water into free water adversely affect the sensorial quality of high moisture foods (e.g. dumplings). These ageing effects should be minimized by the use of appropriate additives for that purpose.

Radiation pasteurization is particularly advantageous as the final processing step, applied to the packed and sealed prepared meal. Nonetheless, the 'bioburden' of the ingredients in the early stages of meal preparation can most often be reduced by alternative methods, which may be advantageous to specific foods. This attitude includes a pretreatment of radiation disinfection of specific ingredients of high bioburden. Thus, the radiation dose at the final stage, which affects all the ingredients, can be substantially lowered, resulting in improved sensorial quality and, just as important, economic profitability.

The efficacy of radiation treatment should be tested/validated under actual commercial conditions, since microbial growth might be greatly facilitated under more or less abusive temperature conditions which may frequently occur in the food chain and during home storage prior to consumption.

Suitable packaging material and processes, as well as adequate storage conditions, are prerequisites for successful deployment of this technology.

Regulatory approval for new radiation pasteurized prepared meal products is critical, both legally and economically, since it is crucial to allow their market testing and consequent marketing as soon as their development is complete. Hence, it is strongly recommended that care be taken of all legal aspects of the petitions for new products as soon as completion of their development seems viable. New concepts, such as multistage irradiation (raw material decontamination and end product treatment), should receive proper consideration by the regulatory authorities.

In conjunction with technological development, market and economic studies are crucial to successful commercialization of irradiated prepared meals.

Alongside the continuing research and development in this field, relevant agencies should disseminate information and assist technology transfer to facilitate the proper utilization of the knowledge gathered during CRPs such as this one. They could also offer neutral and precompetitive support of such knowledge for the common good of all the relevant stakeholders, including regulators of food irradiation. In such meaningful technology transfer, comparisons of relative advantages and/or disadvantages of alternative technologies should always be considered.

It is essential to provide a solid scientific foundation for a wider application of the knowledge gained from this CRP. Hence international cooperative research efforts are needed for further implementing the technology.

6. PUBLICATIONS

A list of the publications to date resulting from the research work carried out under this CRP is given in Annex I.

SAFER PREPARED MEALS FOR IMMUNOCOMPROMISED PATIENTS AND THE GENERAL CONSUMER BY GAMMA IRRADIATION

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Abstract

Ready-to-eat meals are commonplace nowadays in urban life. Many of them, minimally processed, could convey foodborne pathogens likely to cause diseases in the consumer, which is of concern to the normal population and even more so to immunocompromised patients. The feasibility of attaining microbiological decontamination at pasteurization levels of such foods by gamma irradiation was studied. Typical Argentine dishes were chosen after market surveys: cannelloni in tomato sauce, tomato and carrot salad with boiled egg, empanada, fruit salad in gelatin jelly with white cheese, ham and cheese sandwich, chicken and vegetable pie, custard, and bread pudding, in different packaging. Microbiological profiles of the meals were obtained and challenge tests with *Listeria innocua* or *Salmonella enteritidis* were performed to determine the minimum radiation dose to be applied in each food so as to attain a 6 log cycle reduction of pathogen counts. Preliminary sensory evaluations, out of panel, were carried out to determine possible evident sensory alterations due to the irradiation treatment. Then, a greater number of samples were irradiated at the minimum radiation dose and at a maximum dose equal to or less than twice the minimum, at the ^{60}Co semi-industrial facility of the Ezeiza Atomic Center. Microbiological and sensory analyses by a consumer panel were performed on control and irradiated samples throughout storage

life at refrigeration temperatures. A whole irradiated lunch, composed of salad, empanadas and fruit salad, was sampled by 44 immunocompromised patients at a hospital. The composition and adequacy of this lunch was designed by nutritionists. Results showed that it was feasible to attain the proposed decontamination goal without significantly impairing the sensory quality. Shelf life was almost tripled in irradiated samples. Immunocompromised patients enjoyed the irradiated lunch and requested it and other dishes to be made commercially available.

1. INTRODUCTION

The present lifestyle in cities leads to little time for cooking, so many ready-to-eat meals are available in supermarkets. Among them, canned foods do not fulfil the present preference for freshness and nutritional value which the consumers demand. Others are stored under frozen or refrigerated conditions, most of them being minimally processed, which enhances the probability of conveying foodborne pathogens. This is of particular importance to the immunocompromised patients, whose natural defences against diseases are low.

Data from the Regional System of Epidemiologic Watchfulness (SIRVETA), developed by the Panamerican Institute for Food Protection and Zoonoses (Pan American Health Organization), indicate 152 foodborne disease outbreaks in Argentina between 1993 and 2002, representing 3309 ill persons and four deaths. The corresponding figures for the whole region (Latin America and the Caribbean) are 6324 of such outbreaks, 228 579 ill persons, 314 deaths. It must also be recalled that only a few per cent of cases are generally reported.

In Argentina, the National System of Epidemiological Watchfulness (SINAVE) [1] reported 67 outbreaks during 2001, with 1237 ill persons involved. These outbreaks corresponded to 15 geographical regions, out of a total of 35. Homes were the places with the biggest prevalence for these outbreaks to take place (63.3%). The remaining 36.7% of outbreaks happened at collective places (institutions, restaurants, community canteens). The agents responsible were, in most cases, *Salmonella* spp. and *Escherichia coli*, followed, not very closely, by *Staphylococcus aureus* and *Clostridia*. The main foods causing this were, in decreasing order, various meats, prepared meals, water, milk products, filled pasta and eggs.

Few data are available to demonstrate the effectiveness of irradiation to improve the microbiological safety and quality of many types of prepared meals currently being marketed either under ambient, chilled or frozen conditions. A previous study in Argentina [2] showed promising results for

some irradiated ready-to-eat meals for immunocompromised patients, which would allow their diets to be enriched with fresh produce or minimally processed foods, more nutritious and palatable but usually forbidden because of the risk of foodborne diseases. Some conclusions of that work were that the tested variety of meals should be widened. Also, and as an example, some studies carried out at the Clinical Hospital José de San Martín in Buenos Aires [3] indicate that because of the slow distribution of lunch or supper within the institution, the meals arrive at the patient's bed lukewarm or even cold, which enhances the probability of microbial growth.

The response of some ready-to-eat meals, widely consumed by the Argentine population, under ionizing radiation processing was studied in this work. Their usual commercial manufacture includes several steps in which original or cross-contamination with pathogenic microorganisms, provided either by the raw food materials, the environment or the personnel, can occur [4, 5]. Ionizing radiation has proven effective in controlling pathogen risk, which both protects the consumer and benefits the producer owing to shelf life extension [6–18]. This is especially important for the immunocompromised population [19–27].

Among the list of known non-spore pathogenic microorganisms likely to contaminate these ready-to-eat foods, *Listeria* was generally chosen for challenge tests because of its being widespread, feasible of growing at refrigeration temperatures, and because it is possibly the most radiation resistant in this group. In some other cases and according to the food product, *Salmonella* was chosen instead.

Besides the microbiological safety, the chemical quality of these irradiated ready-to-eat meals had to be evaluated. According to the literature, nutritional stability at these radiation doses was considered to be fairly well established [28–33], but as organoleptic changes could take place sensory analyses were performed to indicate potential consumer acceptability in the future.

2. MATERIALS AND METHODS

The main brand supermarkets in Argentina, along with various food chains and retailers, were visited in order to find the most popular meals offered which could benefit from irradiation treatment. At least four varieties of each product, sourced from different suppliers, were initially sampled by a panel of six persons in order to choose the best manufacturer. Selection was carried out on the basis of parameters such as food quality, packaging and radiation dose, so as to minimize sensory damage after treatment. Selected meals and their characteristics are summarized in Table 1.

TABLE 1. CHARACTERISTICS OF SELECTED MEALS

Meal	Ingredients	Packaging	Manufacturer	Comments on manufacturing and cooking	Estimated shelf life under refrigeration
Cannelloni	Water, wheat flour, cooked veal, fresh spinach, grated Provolone cheese, pasteurized egg, sodium chloride, sodium glutamate, ground nutmeg Sauce: canned tomato puree, fried onion, dehydrated marjoram	Polystyrene containers with lid	Factory of a national good quality brand which distributes its products widely in Argentina. They were specially manufactured for this experience without the addition of potassium sorbate as a preservative	Samples were cooked inside their closed packaging in a General Electric microwave oven (model Je 1831) for 6 min at level 4	5 d (commercial presentation, without sauce)
Salad	Raw grated carrot, raw whole cherry tomatoes, hard boiled egg	Polypropylene trays, covered with PVC film	Nutrition students at the laboratory	Good manufacturing practices were performed	2 d
Fruit salad	Fresh apples, pears, orange juice, gelatin powder, strawberry flavour, Punzo Red colourant, water, white cheese	Polystyrene containers with lid	Nutrition students at the laboratory High quality commercial brand	Raw Granny Smith apples and Packam pears, peeled and cut into cubes, were soaked in fresh orange juice	3 d

TABLE 1. CHARACTERISTICS OF SELECTED MEALS (cont.)

Meal	Ingredients	Packaging	Manufacturer	Comments on manufacturing and cooking	Estimated shelf life under refrigeration
Sandwich	Water, wheat flour, margarine, vegetable oil, egg yolk, cooked ham, Edam cheese	Polystyrene tray covered with PVC film	Supermarket	Three thin, spongy, flat white wheat bread layers, which had been covered on one side with a fat mixture made of mayonnaise and margarine, and contained a slice of cooked ham and another of Edam cheese between layers	2 d
Pie	Pasta discs: water, wheat flour, margarine Filling: onion, tomato, milk, chicken broth, chicken breast, fresh capsicum, sodium chloride, sodium propionate	Pie portions were packaged into aluminium foil recipes with lid	Supermarket	Filling placed between two thin, wheat flour pasta discs, 25 cm diameter, one at the base and the other on the top. The wrapping of these discs, to avoid filling losses, is handmade. They are usually sold already cooked Reheated in gas oven at 220°C for 6 min, lid half opened	2 d

TABLE 1. CHARACTERISTICS OF SELECTED MEALS (cont.)

Meal	Ingredients	Packaging	Manufacturer	Comments on manufacturing and cooking	Estimated shelf life under refrigeration
Custard	Milk, sugar, egg, vanilla essence	Aluminium foil covered with PVC film	Supermarket		2 d
Bread pudding	Bread (preferably one day old), milk, sugar, egg, grated lemon peel, vanilla essence	Portions were packaged into polystyrene container with lid	Pastry commercial chain		4 d

Irradiation was carried out on one-day-old samples, at the semi-industrial ^{60}Co facility of the Ezeiza Atomic Center, using 1.1×10^{10} MBq of activity, at a dose rate of 0.15 kGy/min. Doses were measured with silver dichromate [34]. Samples were placed into polystyrene boxes containing cooling bags to ensure maintaining refrigeration temperature during treatment. These boxes were rotated after half the irradiation time to ensure a homogenous dose distribution. Some other samples were kept as controls and not irradiated. Every sample was stored at 5 ± 2°C and 60 ± 8% RH along storage time.

The microbiological profile of the selected meals was evaluated on at least two production batches; results are the average of ten replicates. For every meal, 100 samples were investigated to evaluate survival of a specific inoculated microbial pathogen at increasing radiation doses (0, 1, 2, 2.5, 3 and 4 kGy), and to detect obvious differences attributable to the treatment by a preliminary sensory evaluation of samples irradiated at the estimated minimum (3 kGy) and maximum doses (6 kGy). The inoculated microorganisms in these challenge tests were:

- *Listeria innocua* (for *Listeria monocytogenes*) in cannelloni, according to Notermans et al. [35].
- *Salmonella enteritidis* in salad, empanadas and fruit salad. An inoculum of 10^8 *S. enteritidis* cells/mL was prepared according to the McFarland scale. Turbidity was adjusted to 0.5 and the suspension diluted to 10^6 cells/mL. One millilitre of the suspension was inoculated into each meal with a sterile syringe and another millilitre was spread on plate count agar to check inoculum recovery [36, 37].
- *L. monocytogenes* ATCC 7973 in sandwiches and chicken pie. Single path *Listeria* was used as a screening method, according to the FDA [38] and also the ELISA LISVIA Tecra® test, according to the USDA [39, 40].
- *Salmonella typhimurium* in custards, according to FIL standards [41]. A batch of concentration with 10^7 cells/mL was inoculated with a syringe into the middle of each sample.
- *L. monocytogenes* ATCC 15313 and *S. enteritidis* ATCC 13076 in bread pudding. In this case, a single colony was used to inoculate fresh tryptic soy broth (Difco) for each experiment, which was incubated for 18 h at 32°C with rotational agitation. Cell density was typically 10^8 CFU/mL. One millilitre of this solution was used to inoculate about 100 g of the product with a syringe. The inoculated products were stabilized in a refrigerator (4°C) for 18 h before irradiation.

Replicates of microbiological analysis ranged between 3 and 10, and the meal sampling for these tests ranged between 5 and 10. A second experiment

consisted of irradiating new samples at the lowest dose used in the first experiment in order to obtain a 6 log cycle reduction in pathogen counts (D_{min}), and at twice that dose (D_{max}). Non-irradiated samples were kept as controls. About 300 samples were analysed at this stage for each meal. Microbiological analysis required by the Argentine Alimentary Code was performed on control and irradiated samples during storage, including assessment of total bacterial counts, moulds and yeasts, spore-forming anaerobes (required only for filled pasta such as cannelloni), *Salmonella* spp., *S. aureus*, coliforms and faecal coliforms, according to ICMSF, USDA, FSIS, FDA and FIL methods [36–43].

These samples were sensorially evaluated by a ~50 member 'healthy' consumer panel twice during their estimated shelf life. Panellists comprised about 40% men and 60% women, with ages ranging from 25 to 65 years, 60% university educated, 40% secondary school education, working at the Ezeiza Atomic Center. The evaluated attributes were, in general, aroma, aspect, colour, flavour, texture and general acceptability, with a nine point hedonic scale ranging from 'like extremely' to 'dislike extremely'. Results were statistically analysed by the Dunnet test, $p < 0.05$ [44].

Forty-four immunocompromised patients also tasted a whole irradiated lunch, consisting of salad, empanadas and fruit salad in gelatin. These patients were: 70% women, 30% men, ages ranging between 18 and 75 years, their conditions being: pregnancy: 30%, cancer 25%, high dose corticoid treatment 16%, blood albumin <3.5 g/dL 16%, neutropenia 11%, HIV+ 2% [45, 46]. Meals comprising the immunocompromised patients' lunch were selected after a survey at the hospital; there was a definite desire for colourful, juicy, fresh, raw products, or for others with some kind of filling such as empanadas, usually not allowed them owing to the risk of undercooking. Meals usually served to immunocompromised patients in this hospital, as in many others in Argentina, do not come from an external catering service but are prepared in the hospital kitchen.

Sensory evaluation by the consumer panel was performed on days 2 and 15 for cannelloni, and for the rest of the meals, on days 2 and 8 after irradiation. Control samples were only evaluated on the first analysis date so as to avoid microbiological risk. Immunocompromised patients evaluated the meals on days 2 and 8, and then only samples irradiated at D_{min}.

3. RESULTS AND DISCUSSION

Microbiological profiles of the selected meals are shown in Table 2. Total bacterial counts were high in empanada, salad, sandwich and chicken pie, even soon after their being manufactured. Moulds and yeasts were very high in the

TABLE 2. MICROBIOLOGICAL PROFILE OF MEALS (CFU/g)

Meal	Total bacterial counts	Mould and yeasts	Total coliforms	Faecal coliforms	*Salmonella* spp. (in 25 g)	*S. aureus* (in 100 g)	Sulphite-reducing *Clostridia*
Storage time under refrigeration: 1–2 d							
Cannelloni	7×10^5	7.1×10^3	40		Absent	Absent	<10
Salad	7×10^6	7.7×10^3	1.9×10^4	4.6×10^2	Absent	Absent	
Empanada	10^8	30	7.3×10^2	<10	Absent	Absent	
Fruit salad	3×10^4	2×10^2	<10		Absent	Absent	
Sandwich	6.3×10^7	1.9×10^6	2.7×10^3	3.1×10^2	Absent	Absent	
Chicken pie	3.4×10^6	<10	<0.3	<0.3	Absent	Absent	
Custard	3.8×10^4	30	<0.3	<0.3	Absent	Absent	
Bread pudding	5×10^5	<50	<10	<10	Absent	Absent	
Storage time under refrigeration: 8 d (15 d for cannelloni)							
Cannelloni	1.4×10^6	1.8×10^5	60		Absent	Absent	<10
Salad	5×10^8	5.1×10^4	2.9×10^5	4.2×10^2	Absent	Absent	
Empanada	3×10^9	3×10^2	3.2×10^3	<10	Absent	Absent	
Fruit salad	5×10^2	7×10^3	<10		Absent	Absent	
Sandwich	3.7×10^7	1.7×10^6	3.5×10^3	1.4×10^2	Absent	Absent	
Chicken pie	2.9×10^6	2.2×10^3	<0.3	<0.3	Absent	Absent	
Custard	1.5×10^4	10	<0.3	<0.3	Absent	Absent	
Bread pudding	1×10^4	1.5×10^3	<10	<10	Absent	Absent	

sandwich, high in cannelloni and salad at the beginning of the storage period and very high at the end of it in almost every meal with the exception of custard. Pathogens were generally absent, although faecal coliforms were found in salad and sandwiches. Coliform counts were high in sandwich, salad and empanada. These results are similar to others reported in the literature [47, 48].

According to the results of survival of the pathogen after inoculation, some radiation doses were chosen as the minimum needed to attain a 6 log cycle reduction (see Table 3). The Argentine Food Code [49] allows a dose dispersion (D_{max}/D_{min}) of up to 2 for radiation doses higher than 1 kGy, but according to results of the preliminary sensory evaluation, in some of the studied meals this had to be lowered due to product sensitivity. The main observed changes were off odours and off flavours in fat-containing meals such

TABLE 3. DOSIMETRIC RESULTS

Meal	Radiation dose (required) (kGy)	Minimum radiation dose (delivered) (kGy)	Maximum radiation dose (delivered) (kGy)	Maximum dose/ minimum dose
Cannelloni	3	2.89	4.57	1.58
	6	6.31	8.98	1.42
Salad	2	2.44	3.65	1.5
	3	3.07	4.43	1.44
Empanada	3	3.40	4.83	1.42
	6	7.02	9.16	1.30
Fruit salad	1.5	1.52	2.20	1.45
	2.5	2.64	3.72	1.43
Sandwich	2.5	2.50	2.91	1.16
	4	4.03	4.65	1.15
Chicken pie	3.5	3.60	4.45	1.23
	5	5.12	6.70	1.30
Custard	3	3.76	5.38	1.43
	4.5	5.75	7.81	1.36
Bread pudding	3	3.01	3.73	1.24
	5	4.84	6.33	1.31

as sandwich, chicken pie and egg yolk (salad), and also in custard; texture losses in salad and fruit salad; browning in fruit salad; colour loss in carrot and egg yolk (salad), and in custard.

Microbiological data of control and irradiated meals can be seen in Table 4. Pathogens were generally absent in every sample. Coliforms were present in salad, empanada and sandwich control samples, and they were sufficiently reduced after the irradiation treatment. The same was observed with moulds and yeasts, which showed high counts in the control sandwich, salad and cannelloni throughout the storage period, and in fruit salad in gelatin at the end of it. Total bacterial counts were reduced by 2–3 log cycles in cannelloni and fruit salad in gelatin, by 3–4 log cycles in salad and custard, and by 4–6 log cycles in sandwich, chicken pie, empanada and bread pudding. These results are in agreement with others reported in the literature [50, 51].

Some microbiological requirements of the Argentine Food Code are, for instance, for filled pasta: *S. aureus*: less than 10 per gram; *Salmonella* spp.: absence in 25 g; sulphite reducing *Clostridia*: less than 10 per gram; moulds and yeasts: less than 100 per gram.

The meals' sensory evaluations by the 'healthy panel' are shown in Table 5. No significant differences due to the irradiation treament were observed in cannelloni, empanada, chicken pie and bread pudding. Instead, significant differences were found in irradiated salad (in texture and general acceptability, and also in flavour in the D_{max} sample), fruit salad in gelatin (texture and general acceptability in the D_{max} sample), sandwich (in flavour and general acceptability, and in moisture and aroma in the D_{max} sample), and custard (in aroma, flavour, texture, general acceptability). Though these differences were statistically different, they were small and scores never fell below the threshold line of 5: 'neither like nor dislike'. It is curious to see that irradiated samples generally received better qualifications at the end of the storage period, probably because by then the panellists had no control sample with which to compare, and also perhaps due to the so-called 'irradiation after-effect' which is more noticeable soon after the treatment, decreasing with storage time.

In irradiated sandwiches, ham darkening was observed, especially in low quality products, as has been also reported in the literature [52, 53]. Sandwiches made at the authors' laboratory, and those purchased at high standard bakeries, gave higher sensory scores. However, as one of the objectives of the present work was to transfer results to the food industry, in spite of having obtained better preliminary sensory scores on high standard quality sandwiches, which are in fact consumed only by a small part of the Argentine population, the best one of those manufactured in supermarkets was chosen for irradiation.

TABLE 4. MICROBIOLOGICAL QUALITY OF CONTROL AND IRRADIATED MEALS

Food	Radiation dose (kGy)	Total bacterial counts (CFU/g)	Moulds and yeasts (CFU/g)	Coliform bacteria (CFU/g)	*E. coli* (CFU/g)	*Salmonella* (in 25 g)	*S. aureus* (in 100 g)
Cannelloni (Note: sulphite-reducing *Clostridia* absent in every sample.)			Storage day under refrigeration: 2				
	0	2×10^5	7.1×10^3	43		Absent	Absent
	3	1.7×10^3	<100	<3		Absent	Absent
			Storage day under refrigeration: 15				
	0	7.5×10^6	1.8×10^5	60		Absent	Absent
	3	5×10^3	7×10^2	<3		Absent	Absent
Salad			Storage day under refrigeration: 2				
	0	3.6×10^6	7.7×10^3	1.9×10^4	4.6×10^2	Absent	Absent
	2	1.4×10^3	<10	<10	<10	Absent	Absent
			Storage day under refrigeration: 8				
	0	2.1×10^7	5.1×10^4	2.9×10^5	4.2×10^2	Absent	Absent
	2	3.3×10^3	<10	<10	<10	Absent	Absent
Empanadas			Storage day under refrigeration: 2				
	0	4×10^7	20	5×10^2	Absent	Absent	Absent
	3	10^2	<10	<10	Absent	Absent	Absent
			Storage day under refrigeration: 8				
	0	2×10^8	1.1×10^2	1.1×10^3	Absent	Absent	Absent
	3	3×10^2	<10	<10	Absent	Absent	Absent

TABLE 4. MICROBIOLOGICAL QUALITY OF CONTROL AND IRRADIATED MEALS (cont.)

Food	Radiation dose (kGy)	Total bacterial counts (CFU/g)	Moulds and yeasts (CFU/g)	Coliform bacteria (CFU/g)	*E. coli* (CFU/g)	*Salmonella* (in 25 g)	*S. aureus* (in 100 g)
Fruit salad			Storage day under refrigeration: 2				
	0	2×10^4	90	10	Absent	Absent	Absent
	1.5	40	30	10	Absent	Absent	Absent
			Storage day under refrigeration: 8				
	0	2×10^3	3.7×10^3	<10	Absent	Absent	Absent
	1.5	10^3	10	<10	Absent	Absent	Absent
Sandwich			Storage day under refrigeration: 2				
	0	10^7	2×10^6	10^4	Absent	Absent	Absent
	2.5	6×10^2	7×10^2	<10	Absent	Absent	Absent
			Storage day under refrigeration: 2				
	0	3×10^7	1.1×10^7	7×10^3	Absent	Absent	Absent
	2.5	2×10^3	8×10^2	<10	Absent	Absent	Absent
Chicken pie			Storage day under refrigeration: 2				
	0	2×10^4	<10	<0.3	Absent	Absent	Absent
	3.5	<10	<10	<0.3		Absent	Absent
			Storage day under refrigeration: 8				
	0	2.9×10^6	2.2×10^3	<0.3	Absent	Absent	Absent
	3.5	<10	<10	<0.3	Absent	Absent	Absent

TABLE 4. MICROBIOLOGICAL QUALITY OF CONTROL AND IRRADIATED MEALS (cont.)

Food	Radiation dose (kGy)	Total bacterial counts (CFU/g)	Moulds and yeasts (CFU/g)	Coliform bacteria (CFU/g)	*E. coli* (CFU/g)	*Salmonella* (in 25 g)	*S. aureus* (in 100 g)
Custard			Storage day under refrigeration: 2				
	0	3×10^2	10	<0.3	Absent	Absent	
	3	10^2	<10	<0.3	Absent	Absent	
			Storage day under refrigeration: 11				
	0	4.7×10^6	10	10	Absent	Absent	
	3	10^2	<10	<0.3	Absent	Absent	
Bread pudding			Storage day under refrigeration: 2				
	0	4×10^5	<50	<50	Absent	Absent	Absent
	3	40	<50	<50	Absent	Absent	Absent
			Storage day under refrigeration:				
	0	5×10^6	<50	<50	Absent	Absent	Absent
	3		<50	<50	Absent	Absent	Absent

TABLE 5. SENSORY ANALYSIS RESULTS ('HEALTHY' CONSUMER PANEL)

Food	Radiation dose (kGy)	Aroma	Aspect	Texture	Juiciness	Flavour	Acceptability
Cannelloni			Storage day under refrigeration: 6				
	0	7.1	7.1	7.3	7.2	7.1	7.0
	3	6.4	7.0	7.1	7.0	6.8	6.9
	6	6.3	7.2	7.4	7.2	6.5	6.8
			Storage day under refrigeration: 15				
	3	7.0	7.1	7.4	6.9	7.1	7.2
	6	6.4	7.1	7.6	7.1	7.0	7.0
Empanada			Storage day under refrigeration: 2				
	0	7.0	7.0	7.0	6.6	7.0	7.0
	3	7.2	7.3	7.0	6.5	7.1	7.2
	6	7.1	7.2	7.0	6.8	7.1	7.1
			Storage day under refrigeration: 9				
	3	6.9	7.0	7.1	6.6	7.2	7.1
	6	7.0	6.9	7.1	6.4	7.3	7.2
Chicken pie			Storage day under refrigeration: 4				
	0	7.2	7.6	7.1	6.2	7.3	7.3
	3.5	7.0	7.3	7.0	6.3	7.1	7.0
	5	6.9	7.3	6.9	6.2	7.1	7.0
			Storage day under refrigeration: 9				
	3.5	7.3	7.3	7.3	6.7	7.5	7.4
	5	6.9	7.2	7.0	6.4	7.3	7.1
Sandwich			Storage day under refrigeration: 4				
	0	6.9	7.2	7.2	7.0	7.7	7.5
	2.5	6.8	7.0	7.0	6.7	6.8[a]	6.7
	4	6.4[a]	7.0	6.8	6.7	6.8[a]	6.5[a]
			Storage day under refrigeration: 7				
	2.5	7.0	7.0	7.1	6.7	7.0	7.2
	4	6.9	7.1	7.2	6.7	6.9	7.0

TABLE 5. SENSORY ANALYSIS RESULTS ('HEALTHY' CONSUMER PANEL) (cont.)

Food	Radiation dose (kGy)	Aroma	Aspect	Texture	Juiciness	Flavour	Acceptability
Salad			Storage day under refrigeration: 2				
	0	6.6	7.4	7.4	7.4	7.3	7.3
	2	6.5	7.2	6.9[a]	7.4	6.5[a]	6.8[a]
	3	6.6	7.5	6.8[a]	7.4	6.8	6.8[a]
			Storage day under refrigeration: 7				
	2	6.8	7.2	7.2	7.4	7.0	7.2
	3	6.8	7.1	7.0	7.3	6.9	6.9
Fruit salad			Storage day under refrigeration: 2				
	0	7.2	7.4	7.5	7.6	7.3	7.6
	1.5	7.2	7.5	7.5	7.3	7.5	7.5
	2.5	7.2	7.2	7.4	7.2[a]	7.2	7.2[a]
			Storage day under refrigeration: 8				
	1.5	7.3	7.4	7.4	7.1	7.4	7.3
	2.5	7.3	7.2	7.3	7.0	7.4	7.2
Custard			Storage day under refrigeration: 3				
	0	6.3	6.6	7.0		7.1	7.0
	3.8	5.6[a]	6.1	5.7[a]		6.0[a]	5.8[a]
	5.8	5.6[a]	6.0	5.8[a]		6.4[a]	6.2[a]
			Storage day under refrigeration: 11				
	3.8	6.4	6.0	6.4		7.0	6.6
	5.8	6.3	5.8	6.4		6.9	6.7
Bread pudding			Storage day under refrigeration: 4				
	0	6.9	6.7	5.7		6.5	6.1
	3	7.1	6.9	5.9		6.7	6.4
	5	6.8	6.8	6.1		6.6	6.3
			Storage day under refrigeration: 10				
	3	7.1	6.8	6.0		6.9	6.7
	5	7.0	6.8	6.5		6.8	6.8

[a] Significantly different from control, $p < 0.05$.

It is interesting to compare the different behaviours of custard and bread pudding, their compositions fairly similar except for the wheat flour in the pudding, which lowered water content significantly. A higher water content, which allows more radiolytic reactions through the secondary irradiation effect; a higher fat content; the presence of radiosensitive chemical groups such as sulphur compounds in egg and milk, among other characteristics, could have contributed to acceptability differences between these two products. Other aspects to be noticed are that the more sensorially radioresistant meals were in general those which had been previously cooked, probably because texture losses due to irradiation were lower than those previously caused by heat.

In every case, the close correlation between results obtained through the consumer 'healthy panel' (50 members) and the preliminary sensory evaluation panel (6 members) suggested that the latter is a useful, simpler tool for screening coarse sensory effects on irradiated foods.

Sensory evaluation of the meals tasted as an irradiated lunch by the immunocompromised patients can be seen in Fig. 1. Scores were very good, and higher than those afforded by the consumer 'healthy' panel, probably due to the fact that these patients usually long for meals such as these, with or without fillings, which are forbidden to them owing to the risk of foodborne diseases. It should also be mentioned that changes in 'taste' these patients sometimes suffered due to mouth and gastric mucosa damage did not impair their evaluation of these irradiated meals.

Most patients showed enthusiasm about this experience. One of them, a man who had suffered a kidney transplant, delayed his discharge from the hospital in order to be able to taste the irradiated meals. A woman accepted the trial immediately as she was very fond of salads and had been denied them for the previous three months.

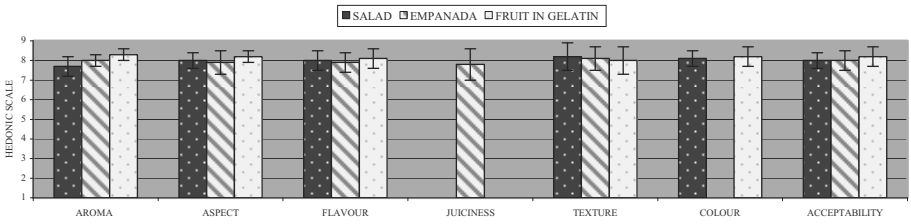

FIG. 1. Sensory evaluation used for irradiated meals, as assessed by immunocompromised patients.

Some of these patients' comments after eating the irradiated lunch were:

"This is a very important meal, moreover because it relates to ill people. I hope this chance will always exist… and that this food could be available all over the world and especially in every Argentine region."

"It lacks quantity. It tastes good; I do not perceive differences with non-irradiated products."

"Very good and very tasty."

"Thank you for informing us of this kind of food preservation method."

"Great. Thank you for looking for solutions to improve our health. Fruits keep [their] typical odour and flavour. Everything was tasty."

"This should be the true food for the ill."

"These are very well presented meals."

"The dessert has the natural fruit flavour."

"At first sight this lunch created a good impression. This new way of [treating] food with ionizing energy seems to me a fantastic idea, and I wish it could be economically feasible, and available to all the community."

"Everthing [was] very tasty."

"I like it because it looks fresh."

"It was very interesting to me that food exposure to gamma irradiation did not change their aspect or taste."

"The packaging kept empanadas still hot, which is very important for ill people, because many times food [is served] cold. Very colourful and agreeable."

"Portions are very generous, presentation is very good, the young ladies who invited (us) to try it showed great courtesy in informing us. Thank you and continue with this work."

"I think this is a good experience for neutropenic persons, and to be able to incorporate more and more foods. They neither lose taste nor colour; I like it to have them available in supermarkets or food services."

Some nutritional aspects of this lunch were evaluated, as well as estimated vitamin losses due to the irradiation treatment, which turned out to be small and similar to those caused by other preservation treatments, such as heat, or even storage [54, 55].

A great amount of discussion of this work was performed in scientific meetings, conferences, expositions, and press and television releases. The informative activities were very valuable in highlighting the whole food irradiation issue, particularly in a country such as Argentina where regulatory authorities appear reluctant to go ahead with further clearances, and sometimes still doubt its wholesomeness. These results are reported in various theses [56–59] and publications [45, 46].

The patients' attitude towards the irradiated lunch deserves special mention. They not only afforded the highest sensory qualifications to the meals, but were also extremely receptive to food irradiation and very grateful to the young nutritionists who cared for their welfare.

4. CONCLUSIONS

Gamma irradiation of the studied ready-to-eat meals rendered microbiologically safe and sensorially acceptable products for at least 8 storage days at refrigeration temperatures, which implies a tripling of shelf life. In cannelloni, shelf life was extended at least to 15 d. Therefore, it was shown that ionizing radiation can be applied not only to simple food systems such as single commodities but also to complex ones such as some whole ready-to-eat meals.

The applied radiation doses were sufficient to reduce 6 log cycles of the most radioresistant microbial pathogens (excluding spore forming bacteria and viruses) that could be present and which cause diseases in the consumer.

A 6 log cycle reduction is considered to be an overestimation of the security margin, as the natural incidence of these microorganisms in food is much lower.

The minimum radiation dose (D_{min}) should in this case not only be enough to fulfil the microbiological goal but also not to exceed the sensory threshold of the most radiosensitive ingredient in the meal. In some cases, a product reformulation or separate irradiation of the meal components might be advisable. This also applies to the maximum dose (D_{max}) that the product would absorb in an industrial treatment, which is generally accepted in most countries' regulations as being up to twice the D_{min} value. Under the experimental conditions of this study, it seemed preferable to lower the D_{max}/D_{min} ratio to protect sensory quality, and so it was chosen to be between 1.4 and 1.6 for most of the meals studied.

Regarding the experience with immunocompromised patients, it was concluded that ionizing radiation, in combination with good manufacturing practices and refrigeration, improves the food quality as more diversification is allowed, with both nutritional and psychological benefits. The level of microbial decontamination attained would enable the offering of longed-for, unusual meals to those persons, without foodborne disease risk. Besides, nutritionists were satisfied with the results because the shelf life extension due to irradiation would allow, in the future, a reasonable flow of meals to be provided to a hospital by a catering service, for instance, once a week, without the food losing its fresh nutritive condition as a consequence of overcooking or freezing.

This was the first approach carried out in Argentina with respect to immunocompromised patients and food irradiation. Much more work should be undertaken to widen the meal variety and to explain this method to other patients, health institutes, catering services and supermarkets. It should be noted that many immunocompromised persons lead a fairly 'normal' life when out of hospitals. The collaboration between food irradiation researchers and nutritionists is considered essential. It would also be desirable to advance regulatory aspects related to this activity.

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IRRADIATION OF PREPARED MEALS FOR MICROBIOLOGICAL SAFETY AND SHELF LIFE EXTENSION

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Abstract

Fourteen international ready meals prepared under the approved hazard analysis critical control point (HACCP) plan and two Ghanaian ready meals, waakye (co-boiled rice and cowpeas served with gravy, minimally processed vegetable salad, hydrated gari, fried fish and macaroni) and jollof rice (rice cooked in tomato sauce and served with gravy and beef tripe), were investigated with the view to enhancing microbiological safety and extending shelf life under chilled conditions. The microbiological count of the complete waakye meal exceeded the microbiological standard. The microbiological counts on meals prepared under the HACCP plan and the jollof rice meals were within the microbiological standards. The D_{10} values for potential pathogens on waakye were 0.271 kGy for *Escherichia coli*, 0.325 kGy for *Salmonella aureus* and 0.440 kGy for *Salmonella* spp. while the D_{10} values on jollof rice meal were 0.173 kGy, 0.260 kGy and 0.285 kGy, respectively. Challenge tests with the pathogens on one of the HACCP meals (poached chicken) or jollof rice suggested that the 3 kGy dose was sufficient for the elimination of the pathogens to ensure the microbiological safety of the meals and extended their shelf life under chilled storage for 28 days without significant effects on their sensory quality. Doses of 1 and 2 kGy did not affect the sensory quality of the rice and chicken/gravy but boiled carrots were unable to withstand a dose of more than 1 kGy.

1. INTRODUCTION

Current trends, in developed countries in particular, suggest an increasing demand for convenience foods such as ready meals and those that require minimum preparation before consumption. In Ghana, the trend is the same with 'street foods' (ready-to-eat meals sold in the informal sector), which play a major role in meeting the nutritional needs of the people [1, 2]. In most developing countries, including Ghana, these ready meals are prepared on a daily basis. Challenges facing ready meals are largely related to shelf stability and microbiological safety. Over 90% of ready-to-eat meals prepared in Ghana

are marketed under ambient conditions and have a shelf life of less than 12 h. The hygienic limitations of street foods in Ghana and elsewhere have been recognized and reported [3–7]. Food service outlets have been reported to harbour varying levels of indicator organisms, sometimes as high as 10^9 CFU/g [5, 8, 9]. Microorganisms of public health concern isolated from these foods included faecal *Streptococci*, coagulase-positive *Staphylococcus aureus* and faecal coliforms, notably *Escherichia coli* and *Shigella flexneri*. Investigations have demonstrated that these cook–chill meals are potential sources of pathogenic bacteria and have been implicated in foodborne disease outbreaks [3–7].

The preparation of meals based on the hazard analysis critical control point (HACCP) approach has the potential to enhance microbiological safety provided the recommended post-processing handling instructions are also complied with to control the multiplication of surviving pathogens. The use of combination treatments involving irradiation in order to eliminate pathogens is critical to ensure the microbiological safety of ready meals. The efficacy of irradiation in controlling pathogens and enhancing food safety has long been established. However, there is a need to develop irradiation protocols for specific ready-to-eat meals in order to ensure not only their microbiological safety but also their acceptability from an organoleptic viewpoint.

The overall objective of this research was to evaluate the effectiveness of irradiation as a method to ensure microbiological safety and extend the shelf life of prepared meals, stored either under chilled or frozen conditions, and to evaluate the sensory quality of the treated products.

2. MATERIALS AND METHODS

2.1. Ready meals and their major components

The following meals were studied:

(a) Fourteen ready meals prepared under the HACCP plan;
(b) Waakye (co-boiled rice and cowpeas served with gravy, minimally processed vegetable salad, hydrated gari, fried fish and macaroni);
(c) Poached chicken meal (poached chicken, boiled rice, gravy and carrots);
(d) Jollof rice (rice cooked in tomato sauce and served with gravy and beef tripe).

2.2. Physicochemical analysis

The pH, water activity, peroxide value, percentage of free fatty acids were determined using standard methods [10].

2.3. Microbiological analysis

2.3.1. Preparation of meal substrate portions

Meal portions of 20 g were prepared by homogenizing for 1 min using a stomacher homogenizer (Mix2, AES Laboratoire, France).

2.3.2. Enumeration and identification tests

A 10 g homogenized meal sample was added to 90 mL peptone water (1% peptone water plus 0.5% NaCl) and stirred on a mechanical shaker (Junior Orbit Shaker, Lab-Line Instruments, United States of America) for 30 min. Standard methods [11] were used in determining the microbiological indices: for total bacteria count (TBC), plate count agar (Oxoid, United Kingdom), incubated at 36°C/48 h; for general coliform count, eosin methylene blue agar (Difco, USA) or violet red bile agar incubated at 36°C/48 h; for *E. coli* count, eosin methylene blue agar (Difco) at 37°C/48 h; for *S. aureus* count, Baird Parker agar (Difco) at 37°C/48 h; for *Salmonella* count, xylose lysine deoxycholate agar (Difco) at 37°C/48 h; for *Salmonella/Shigella,* selective agar (Oxoid) incubated at 37°C/48 h; and for mould and yeast count, Oxytetracycline (0.01%) glucose yeast agar (Merck, Germany) at 28°C/72 h. The enumeration of survivors was done using a colony counter (Stuart Scientific, UK).

Representative colonies from the plate counts were purified by subculturing and identified using morphological characteristics and standard biochemical tests. The tests used were Gram stain, catalase, oxidase, motility, nitrate, carbohydrate fermentations, triple sugar iron and the IMViC tests with reference to Biochemical Tests for Identification of Medical Bacteria [12].

2.3.3. Preparation of inoculum of test pathogens

Cultures of test pathogens, *E. coli*, *S. aureus* and *Salmonella* spp. used for the study were isolated from some locally prepared ready meals. The cultures were stored on eosin methylene blue agar (Difco), Baird Parker agar (Difco) or xylose lysine deoxycholate agar (Difco) at 3–5°C, before they were reactivated by incubation at 37°C for 24 h and used for preparation of inocula. The inocula

were standardized to a concentration of 10^7 CFU/mL by the methods of serial dilution, pour plate and the use of a haemocytometer.

2.3.4. Radiation sensitivity tests

A 5 mL suspension of each test pathogen (population of 10^7 CFU/mL) was added to 25 g portions of the homogenized meals in polythene bags and sealed. The inoculated samples were stored at 3–5°C for 24 h to allow test pathogens to adjust and then treated with irradiation doses of 0.10, 0.30, 0.45, 0.60, 0.75 and 0.85 kGy. Both the controls and irradiated meals were analysed for surviving test pathogens. Three independent experiments were conducted.

2.3.5. Challenge testing experiments

A 5 mL suspension of each test pathogen was added to 25 g of the homogenized meal in a polythene bag and sealed. The inoculated meal samples were stored at 3–5°C for 24 h to allow the test organism to adjust to the substrates (meals). The inoculated meal samples were subsequently treated with irradiation doses of 2 and 3 kGy. After irradiation, both the control and irradiated meals were stored in a refrigerator for 28 d (3–5°C). Enumeration of surviving cells of *E. coli* and *S. aureus* was carried out at 0, 7, 14, 21, 28 d of chilled storage. Three independent experiments of two replicates each were conducted.

2.4. Sensory tests

Irradiated and non-irradiated meals were stored at 3–5°C and at predetermined intervals the meals were subjected to sensory tests using trained panellists. For the poached chicken, the meals were treated with 1.0 or 2.0 kGy and at the appropriate intervals during storage, 24 consumer panellists used triangle, hedonic and acceptability tests for the detection of differences between, and preference for, the sensory quality of irradiated and non-irradiated meals. In the case of the jollof rice, the meals were irradiated with 1.5 or 3.0 kGy and, using descriptive tests, 15 trained panellists determined the effect of irradiation and storage on selected sensory indices of the three components: rice (colour, texture, taste), gravy (flavour, aroma) and beef tripe (chewiness, fibrousness). For each attribute, judgement was made by placing a distinct vertical line across a given horizontal line. In all instances, the meals were heated in a microwave oven (600 W) before presenting them to the panellists.

2.5. Irradiation

Irradiation of the meals was carried out at the semi-commercial ^{60}Co gamma irradiation facility of the Ghana Atomic Energy Commission at a dose rate of 2.31 kGy/h in air.

2.6. Statistical analysis

Statistical analysis was done using the statistical analysis package of Microsoft Excel™ (Microsoft Corp., USA). The microbial counts (CFU/g) were transformed into logarithms (lg) and the data subjected to regression analysis. The surviving fractions, lg (N/N_0), of *E. coli, S. aureus* and *Salmonella* spp. were calculated and used as relative changes of their actual viable cell counts. The D_{10} values (the dose required to inactivate 90% of a population) were calculated by plotting lg (N/N_0) against dose (D) according to the equation [13]:

$$D_{10} = D/(\lg N - \lg N_0)$$

where

N_0 is the initial viable count;
N is the viable count after irradiation with dose D;
D is the radiation dose.

The linear correlation coefficient (r^2) and the regression equations were calculated.

Panellists' scores were statistically analysed using Triangle, Paired Comparison Tables of Significance or two-factor ANOVA with replication (Microsoft Excel).

3. RESULTS AND DISCUSSION

3.1. Ready meals prepared under the HACCP plan

The meals were held at temperatures between –5 and 0°C for periods ranging up to 5 d (Table 1). The TBCs did not exceed 3.95 lg CFU/g whilst the coliform count did not exceed 2.7 lg CFU/g. For the meal coded HAP-3, no coliform colonies were observed after the stipulated incubation period. The presence of *Salmonellae* spp. and *Shigellae* spp. could not be established on any

of the meals as there was no growth of microbes on the selective SS agar. The results of the microbiological tests suggested that for all 14 HACCP guided prepared meals, the counts obtained did not exceed the microbiological standards for such short shelf life foods (TBC $<10^5$ CFU/g at 36°C/48 h and coliforms $<10^4$ CFU/g at 36°C/48 h). These results demonstrated the important contribution that effective implementation of an HACCP plan in the food industry can make towards improving hygiene quality and enhancing food safety.

Additional information in Table 1 which deserves mention is the observed effects on the microbiological quality of holding the meals at 0 to –5°C. The meals are strictly supplied to clients within 24 h of preparation. However, it was considered relevant to investigate the microbiological quality of some of the meals should they be held for periods longer than 24 h. Meals HAP-10 and HAP-11 are the same, although the latter, which was analysed after holding for 2 d, had lower aerobic mesophilic bacteria counts but higher coliforms than HAP-10, which was analysed without holding. It is noteworthy that the counts obtained for meals which were held for up to 5 d were also within the microbiological standards. Potential pathogens isolated from the meals prepared under the HACCP plan included enterotoxigenic bacteria such as *E. coli, Serratia* spp. and *Klebsiella* spp. The last two species were also isolated from the complete waakye meals. Isolates of such potential pathogens on ready meals have been reported. The results suggested that the implementation of an HACCP substantially reduced microbial counts, although some potential pathogens survived. Although the HACCP plan requires that the ready meals be held at 0 to –5°C in order to suppress growth of survivors, the latter could proliferate during temperature abuse when power outages occur.

There is the need to adopt processes/technologies which have the potential to eliminate potential pathogens from prepared meals and improve their microbiological safety. The potential of irradiation to enhance food safety has long been recognized. However, the effectiveness of the treatment is, among other factors, dependent on the dose applied.

Table 2 shows the radiation sensitivity of three potential pathogens, *E. coli*, *S. aureus* and *Salmonella* spp. The D_{10} values suggested that while 271 Gy was required to reduce a population of *E. coli* by 90%, a higher dose of 440 Gy was required to reduce a population of the *Salmonella* spp. by the same amount. The higher radiation sensitivity of *E. coli* compared with *S. aureus* and *Salmonellae* spp. has been reported. This trend, as well as the fact that radiation sensitivity is dependent on the test medium, have been reported.

TABLE 1. MICROBIOLOGICAL QUALITY OF READY MEALS PREPARED UNDER THE HACCP PLAN

Meal code[a]	Days held at 0 to –5°C	TBC[b] (lg CFU/g)	Coliform count (lg CFU/g)	Isolates
HAP-1	2	3.95 ± 0.03	1.92 ± 0.02	*Pseudomonas* spp., *Alkaligenes* spp.
HAP-2	3	3.84 ± 0.04	0.39 ± 0.12	*E. coli, Enterobacter* spp.
HAP-3	4	1.33 ± 0.04	Not detected	*E. coli, Enterobacter* spp.
HAP-4	2	1.70 ± 0.02	0.15 ± 0.21	*Acinetobacter* spp.
HAP-5	3	3.06 ± 0.03	2.70 ± 0.32	*Acinetobacter* spp., *Serratia* spp.
HAP-6	4	1.54 ± 0.05	1.34 ± 0.11	*Acinetobacter* spp.
HAP-7	4	3.91 ± 0.03	1.78 ± 0.16	*Enterobacter* spp.
HAP-8	4	2.71 ± 0.03	1.63 ± 0.04	*Serratia* spp., *Enterobacter* spp.
HAP-9	5	2.76 ± 0.02	2.11 ± 0.05	*E. coli, Klebsiella* spp., *Acinetobacter* spp.
HAP-10	0	2.73 ± 0.01	0.69 ± 0.12	*Acinetobacter* spp., *Serratia* spp.
HAP-11	2	1.50 ± 0.71	1.42 ± 0.02	*Acinetobacter* spp., *Serratia* spp.
HAP-12	2	1.25 ± 0.10	0.39 ± 0.13	*Acinetobacter* spp.
HAP-13	4	3.45 ± 0.43	0.69 ± 0.13	*Enterobacter* spp., *Klebsiella* spp.
HAP-14	2	2.06 ± 0.03	0.30 ± 0.43	*Enterobacter* spp., *Acinetobacter* spp.

[a] Meal ingredients:
HAP-1: Fish fillet, cheese, sauce, oil, potato, spices, vegetables
HAP-2: Deboned chicken, spices, oil, plain rice, potato
HAP-3: Chicken, spices, sauce, oil, steamed rice, potato
HAP-4: Beef steak, spices, oil, potato, tomato, peppers
HAP-5: Beef steak, spices, sauce, oil, steamed rice
HAP-6 Chicken breast, spices, oil, boiled rice, sauce
HAP-7 Fish fillet, spices, oil, sauce, potato
HAP-8: Chicken, peppers, curry, oil, steamed rice
HAP-9: Fish, spices, oil, potato, sauce
HAP-10: Eggs, salt, oil
HAP-11: Eggs, salt, oil
HAP-12: Lamb, spices, oil
HAP-13: Chicken, spices, oil, vegetables, rice
HAP-14: Biscuit, fruits, cheese, cream.

[b] Mean counts ± SD.

TABLE 2. RADIOSENSITIVITIES OF *E. coli, S. aureus* AND *Salmonella* spp. ON COMPLETE WAAKYE

Pathogen	Regression equation	r^2	D_{10} value (kGy)
E. coli	$Y = -0.560X + 0.167$	0.889	0.271 ± 0.060[a]
S. aureus	$Y = -0.462X + 0.475$	0.988	0.325 ± 0.048
Salmonella spp.	$Y = -0.344X + 0.238$	0.985	0.440 ± 0.064

[a] Mean ± SD of three independent experiments of three replicates each.

TABLE 3. WATER ACTIVITY AND pH OF WAAKYE AND ITS ACCOMPANIMENTS

Sample	Water activity	pH
Complete[a] waakye	0.939 ± 0.001[b]	5.60 ± 0.02
Waakye only	0.936 ± 0.001	7.83 ± 0.01
Fried fish	0.887 ± 0.004	5.80 ± 0.10
Hydrated gari	0.931 ± 0.001	4.35 ± 0.03
Cooked macaroni	0.943 ± 0.001	6.48 ± 0.71
Vegetable salad	0.948 ± 0.001	4.85 ± 0.01
Gravy	0.897 ± 0	5.11 ± 0.01

[a] Waakye, gravy, fried fish, hydrated gari, macaroni and vegetable salad.
[b] Mean ± SD.

3.2. Waakye and other ready meals

3.2.1. Waakye and its accompaniments

The vegetable salad had the highest water activity (0.943) while fried fish had the lowest (0.887) (Table 3). Complete waakye, which consisted of waakye and all its accompaniments, had a water activity of 0.939.

With respect to pH, hydrated gari (4.35) and the vegetable salad (4.85) had relatively lower values than the other meals. It is important to note the significant reduction in the pH of waakye alone from 7.83 to 5.60 when the accompaniments are added. Water activity and pH greatly influence the microbiological quality and shelf life of foods.

TABLE 4. MICROBIOLOGICAL COUNT (lg CFU/g) OF WAAKYE AND ITS ACCOMPANIMENTS

Sample	TBC	Coliform count	Moulds and yeasts
Complete[a] waakye	7.01 ± 0.15[b]	5.88 ± 0.16[c]	3.58 ± 1.47
Waakye only	3.93 ± 0.47	1.19 ± 0.48	Not detected
Fried fish	5.00 ± 1.53	1.27 ± 1.79	1.12 ± 0.66
Hydrated gari	2.34 ± 1.29	1.27 ± 1.79	0.48 ± 0.68
Cooked macaroni	7.49 ± 0.72	6.48 ± 0.71	1.12 ± 0.66
Vegetable salad	7.24 ± 0.69	6.37 ± 0.63	3.26 ± 0.73

[a] Waakye, gravy, fried fish, hydrated gari, macaroni and vegetable salad.
[b] Mean ± SD.
[c] Positive coliform plates of complete waakye indicated the presence of *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter freundii.*

The TBC for complete waakye meals was 7.01 lg CFU/g whilst coliform count 5.88 lg CFU/g. These values are comparable to the counts obtained for macaroni (TBC: 7.49 lg CFU/g, coliform count: 6.48 lg CFU/g) and salad (TBC: 7.24 lg CFU/g and coliform count: 6.37 lg CFU/g). Relatively lower TBC (3.93 lg CFU/g) and coliform count (1.19 lg CFU/g) were obtained for waakye only. The fried fish also had a high coliform count (4.67 lg CFU/g) (Table 4).

Mould and yeast counts were present on complete waakye (3.58 lg CFU/g) and the raw vegetable salad (3.26 lg CFU/g) but could not be detected on waakye alone. From these results, the raw vegetable salad appeared to be the source of moulds and yeasts in the complete waakye meal. *Salmonellae* spp. and *Shigellae* spp. could not be detected on any of the samples. However, the fact that the coliform count (5.88 lg CFU/g) exceeded the microbiological standards for such short shelf life foods (TBC $<10^5$ CFU/g at 36°C/48 h and coliforms $<10^4$ CFU/g at 36°C/48 h) has serious implications on the microbiological safety of the complete waakye meal.

The high coliform count on the meals in Table 4 is noteworthy. Isolates from positive plates for the complete waakye included *Klebsiella* spp., *Enterobacter* spp. and *C. freundii*. The observed high count on the vegetable salad and macaroni (Table 4) and the fact that they reflected the high count for complete waakye deserve further investigation. Uncooked vegetable salads naturally have a high microbial load unless strict procedures, including thorough washing and sanitization, are followed to reduce microbial load, while subsequent refrigeration or chilling is a requirement to control microbial growth. Discussions with, and observation of, waakye processors revealed that the vegetable salads

were washed with potable water, comminuted and displayed together with other accompaniments of waakye, during marketing. There is, therefore, no effective sanitization treatment for the salads. Earlier studies also indicated that accompaniments of waakye, such as salads and pepper sauce, which are minimally processed, had high counts (aerobic mesophilic count $>4 \times 10^3$ CFU/g, *S. aureus* $>3 \times 10^4$ CFU/g and *Bacillus cereus* $>2 \times 10^5$ CFU/g).

A direct observation of waakye sellers also suggested cross-contamination resulting from the use of fingers or the same ladle to serve the salads, cooked macaroni and waakye. Although the use of different ladles would largely prevent cross-contamination among the meal components, this would not prevent the contamination of the complete waakye meal as the results of the current study indicate (Table 4). Isolates from the positive coliform plates of complete waakye included *C. freundii*, *Klebsiella* spp. and *Enterobacter* spp., which have public health implications. The results suggested that the poor microbiological quality of many street foods might result from the combined impact of ineffective sanitary procedures during the preparation of the foods, post-processing contamination and long holding under conditions which enhance proliferation of microorganisms. Improving sanitary conditions under which street foods are prepared and marketed and assisting stakeholders to adopt effective preventive procedures based on the HACCP approach, to convert the raw food ingredients into ready-to-eat meals, are critical towards ensuring food safety and good public health.

3.3. Poached chicken meal

The changes in physicochemical properties during storage of the various components in non-irradiated and irradiated meals are shown in Table 5. The high water activity and the pH range of the meals make them suitable for microbial proliferation. With respect to free fatty acids, the component of most interest is the chicken and gravy because of their relatively higher fat content. The results suggested that the irradiation treatment and chilled storage did not induce fat hydrolysis.

3.3.1. Microbiological quality of poached chicken meal

The TBC of the various meal components is presented in Table 6. Counts obtained for both the irradiated and control meal components on the first day (0) did not exceed 2.53 lg CFU/g. Although counts increased during storage, TBCs for the irradiated meals were lower than the non-irradiated samples. On day 14, only the irradiated rice and irradiated chicken/gravy had TBC which did not exceed the microbiological standard for such ready meals; the

TABLE 5. CHANGES IN pH, FREE FATTY ACIDS AND WATER ACTIVITY OF POACHED CHICKEN MEAL DURING CHILLED STORAGE

Meal component	Treatment (kGy)	Storage time (d)	pH	Free fatty acid (%)	Water activity
Rice:		0	5.8	<0.01	0.937
Control	0	9	5.8	<0.01	0.938
		14	6.0	<0.01	0.938
		22	6.2	<0.01	0.942
Irradiated	1	0	5.9	<0.01	0.940
		9	6.0	<0.01	0.940
		14	6.1	<0.01	0.940
		22	6.1	<0.01	0.946
Carrots:		0	6.3	<0.01	0.943
Control	0	9	4.7	<0.01	0.938
		14	5.3	<0.01	0.953
		22	n.a.[a]	n.a.	n.a.
Irradiated	1	0	6.3	<0.01	0.946
		9	6.3	<0.01	0.946
		14	6.4	<0.01	0.944
		22	n.a.	n.a.	n.a.
Chicken and gravy:		0	5.7	0.03	0.943
Control	0	9	5.3	0.03	0.934
		14	5.7	0.03	0.939
		22	n.a.	n.a.	n.a.
Irradiated	2	0	5.4	0.03	0.944
		9	5.7	0.03	0.941
		14	5.4	0.03	0.936
		22	5.2	0.03	0.939

[a] n.a.: not analysed.

irradiated carrots had deteriorated. At the end of the storage period of 21 d, only irradiated rice had counts (2.18 lg CFU/g) which did not exceed the microbiological standard. These results indicated that irradiation improved the microbiological quality and extended the shelf life of the meals to 14 d.

The sensory quality of control and irradiated poached chicken samples is shown in Table 7.

No significant difference ($p > 0.05$) was observed between the irradiated and non-irradiated meal components (rice, chicken and gravy) on days 0 and 3 during chilled storage. On the 15th day, panellists also detected significant

TABLE 6. EFFECT OF IRRADIATION ON THE TBC IN COMPONENTS OF POACHED CHICKEN MEAL DURING STORAGE AT 3–5°C

Components	Dose (kGy)	Storage time (d)			
		0	9	14	21
Rice	0	2.24 ± 0.26[a]	3.17 ± 1.28	3.53 ± 0.78	5.56 ± 0.62
Rice	1	n.d.[b]	1.50 ± 0.44	n.d.	2.18 ± 0.55
Carrots	0	2.53 ± 0.18	1.87 ± 0.45	5.58 ± 0.05	n.a.[c]
Carrots	1	1.05 ± 0.10	1.43 ± 0.61	n.a.	n.a.
Chicken & Gravy	0	1.68 ± 0.37	3.72 ± 0.54	u[d]	n.a.
Chicken & Gravy	2	1.35 ± 0.15	3.60 ± 0.35		

[a] Mean count: lg CFU/g ± SD (n = 3).
[b] n.d.: not detected.
[c] n.a.: not analysed owing to poor state and offensive odour.
[d] u: uncountable, in excess of 6.00.

differences between the irradiated and non-irradiated chicken/gravy; only the irradiated chicken/gravy was acceptable to the panellists. The irradiated rice and irradiated chicken and gravy were acceptable to the panellists on the 22nd day but the non-irradiated equivalents were rejected. The rejection of the non-irradiated chicken/gravy on the 15th day may be attributed to the high microbial population (Table 6) and the related bad odour. It has been reported that irradiated spicy chicken basil was rejected by panellists after 7 d storage even though TBC did not exceed 10^3 CFU/g. They attributed the rejection of the spicy chicken to objectionable changes in the aroma of irradiated basil. Clearly sensory rejection of foods may not only be due to microbial activity but also to chemical changes.

The results have shown that irradiation doses of 1 kGy for rice and 2 kGy for chicken/gravy effectively controlled microflora, sensory quality and extended shelf life up to 14 d under chilled storage. The observed extension in the shelf life of irradiated poached chicken/gravy and rice is very significant since the recommended maximum shelf life of ready meals at 0–3°C is 5 d. The positive impact of combinations of low dose irradiation and chilled storage on the microbiological quality and shelf life of ready meals has also been reported.

Survival curves of *E. coli* and *S. aureus* were studied as a function of irradiation dose. Figures 1 and 2 show that surviving *E. coli* and *S. aureus* cells decreased with increasing irradiation dose.

TABLE 7. SENSORY SCORES FOR IRRADIATED AND NON-IRRADIATED POACHED CHICKEN AND RICE STORED AT 4 ± 1ºC FOR 22 DAYS

Meal portion	Dose (kGy)	Storage time (d)								
		9			15			22		
		Taste	Colour	Texture	Taste	Colour	Texture	Taste	Colour	Texture
Rice	0	7.2	7.4	6.5	7.4	7.4	7.4	Rejected	Rejected	Rejected
Rice	1	7.6	7.3	6.6	7.3	7.0	7.3	7.3	7.2	7.1
Chicken and gravy	0	7.1	7.5	6.8	3.9	5.3	5.8	Rejected	Rejected	Rejected
Chicken and gravy	2	7.4	7.6	6.9	7.2	6.4	6.6	6.5	7.3	7.5

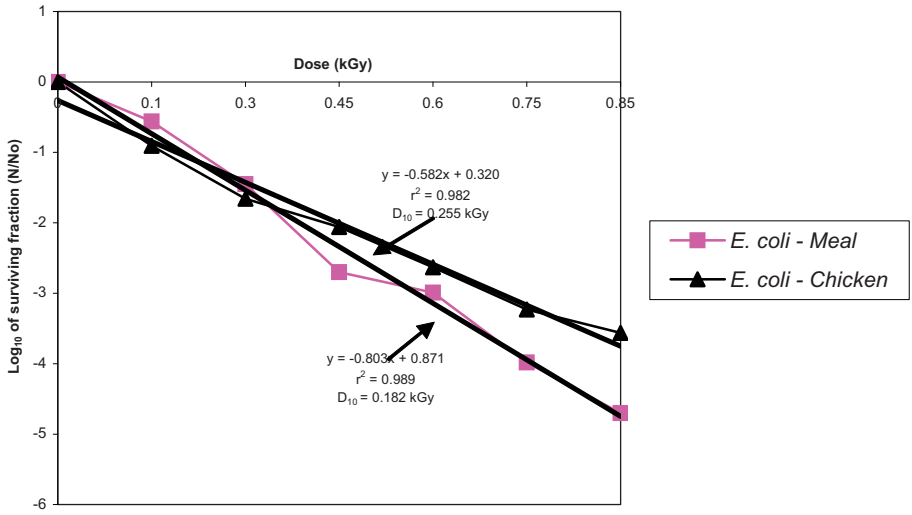

FIG. 1. Radioresistance curves of E. coli.

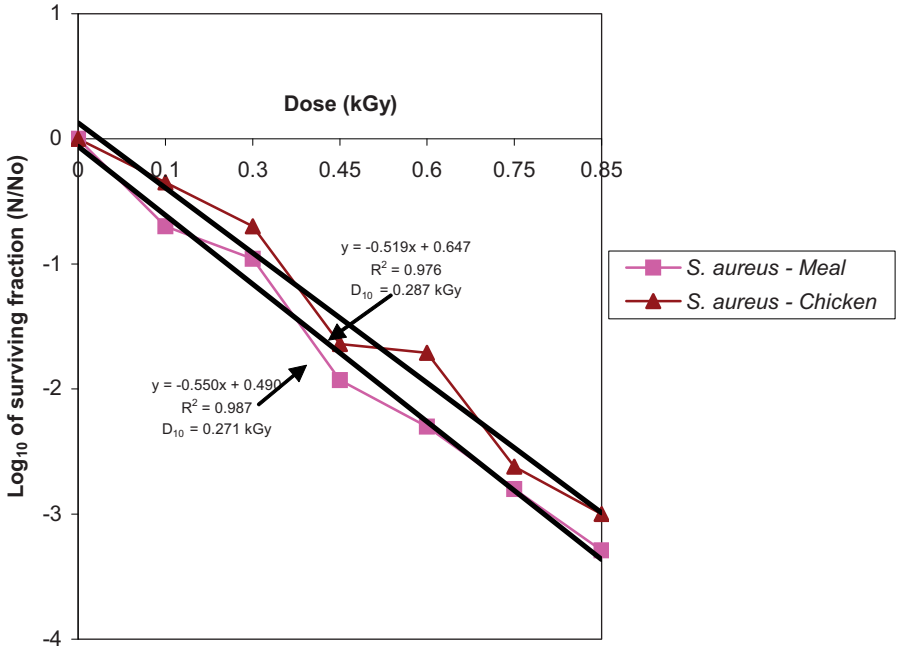

FIG. 2. Radioresistance curves of S. aureus.

TABLE 8. RADIOSENSITIVITY OF *E. coli* AND *S. aureus* ON POACHED CHICKEN MEAL AND MINCED CHICKEN SUBSTRATE

Meal/substrate	Test organism	Regression equation	r^2	D_{10} value
Poached chicken meal	*E. coli*	$Y = -0.803X + 0.871$	0.989	0.182 ± 0.019[a]
	S. aureus	$Y = -0.550X + 0.490$	0.987	0.271 ± 0.004
Minced chicken substrate	*E. coli*	$Y = -0.582X + 0.320$	0.982	0.255 ± 0.003
	S. aureus	$Y = -0.519X + 0.647$	0.976	0.287 ± 0.009

[a] Mean ± SD (n = 2).

As can be seen in Table 8, the calculated D_{10} values of *E. coli* on poached chicken meal and minced chicken substrate were 0.182 kGy and 0.255 kGy, respectively; and those of *S. aureus* were 0.271 kGy and 0.287 kGy, respectively. The linear correlation coefficients of the regression lines were all greater than 0.970 and highly significant ($p < 0.01$), indicating a strong negative linear correlation. The D_{10} values were lower on the poached chicken meal than the minced chicken substrate and values for *S. aureus* were higher than *E. coli*. The calculated D_{10} values of *E. coli* compare well with the reported range of 0.16–0.39 kGy on beef, chicken and other poultry meat by other studies. The calculated D_{10} of *S. aureus* reported in this work also compares well with the reported range of 0.22–0.58 kGy on meat and poultry. The reported D_{10} values of the pathogens on the minced chicken substrate were higher than those on the poached chicken meal.

Viable cells of *E. coli* and *S. aureus* in the non-irradiated poached chicken meal stored at 3–5°C decreased by 1 and 2 log cycles respectively over the 28 d storage period (Table 9, Fig. 3). Though viable cells of *S. aureus* increased slightly by the 7th day, both viable cells of *E. coli* and S. *aureus* decreased gradually to the 28th day (Fig. 3). A dose of 2 kGy reduced viable cells of *E. coli* by almost 8 log cycles such that no surviving viable cells were detected in the 28 d storage period.

A dose of 3 kGy eliminated the viable cells of *S. aureus* (Table 9, Fig. 3). The results showed that populations of *E. coli* and *S. aureus* inoculated into the poached chicken meal remained fairly stable for 7 d, after which period the numbers declined by almost 2 log cycles to between 6.0 and 7.0 lg CFU/g after a 28 d storage period. Other researchers have reported that pathogenic *E. coli* generally survived well in foods at refrigeration temperatures (3–7°C) with a $10^{0.5}$–$10^{1.5}$ CFU/g reduction over 1–5 weeks storage. Similarly, reports have also indicated that though *S. aureus* has a long lag time at temperatures around 7–10°C, it survives and grows very slowly at lower temperatures. A dose of

TABLE 9. EFFECT OF IRRADIATION ON THE GROWTH OF *S. aureus* AND *E. coli* ON POACHED CHICKEN MEAL STORED AT 3–5°C

Micro-organism	Dose (kGy)	Storage time (d)				
		0	7	14	21	28
E. coli	0	8.00 ± 1.05[a]	7.91 ± 1.30	7.77 ± 1.35	7.22 ± 0.94	6.63 ± 1.00
S. aureus	0	8.30 ± 1.03	8.48 ± 0.78	8.21 ± 0.50	7.17 ± 0.82	6.47 ± 1.59
E. coli	2	0	0	0	0	0
S. aureus	2	3.10 ± 0.27	2.27 ± 0.05	1.91 ± 0.63	1.56 ± 0.58	0.62 ± 0.72
E. coli	3	0	0	0	0	0
S. aureus	3	0	0	0	0	0

[a] Mean count (lg CFU/g) of two independent experiments of two replicates each. Mean ± SD (n = 4).

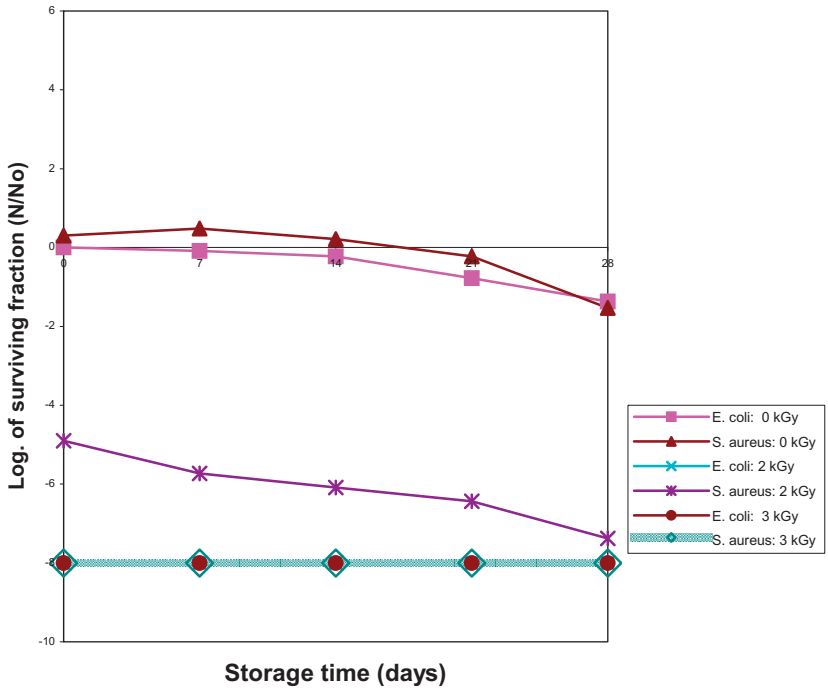

FIG. 3. Survival of E. coli *and* S. aureus *cells in irradiated and unirradiated poached chicken meal stored at 3–5°C.*

2 kGy reduced viable cells by 8 log cycles and completely eliminated *E. coli* from the inoculated poached chicken meals. This finding is clearly supported by the calculated D_{10} value of 0.18 kGy, which indicated that a dose of 2 kGy was adequate enough to render inactivate an initial pathogen population of 10^8 CFU/g. It has also been reported that a dose of 2 kGy reduced viable cells of *E. coli* by 7 log cycles and extended the shelf life of minced beef at 4°C three- to fivefold. The high susceptibility of *E. coli* to gamma radiation observed in this study has also been reported elsewhere. In the case of *S. aureus*, a dose of 2 kGy reduced the initial viable cells by 5 log cycles to 3.10 lg CFU/g in the inoculated poached chicken meal; and this residual population decreased over the 28 d to <1.0 lg CFU/g. This finding is supported by other studies which have indicated that though *S. aureus* is very susceptible to ionizing radiation, staphylococcal enterotoxin is very resistant to irradiation and will not be destroyed by the approved dose used for the treatment of food. In addition, it has been established that *S. aureus* is very resistant to heat and therefore survives cooking in most instances. These facts are very significant since 10^4 CFU/g are sufficient to produce food poisoning toxins. These findings emphasize the need to use methods such as irradiation to eliminate *S. aureus* and therefore prevent proliferation in foods.

In this study, 2 kGy completely eliminated *E. coli* while 3 kGy was required to eliminate *S. aureus* (10^8 CFU/g) and prevent their growth throughout the 28 d storage period. However, considering the fact that both *E. coli* and *S. aureus* are likely to be present in chilled ready meals, 3 kGy is the recommended dose to guarantee the hygienic safety of chilled poached chicken meal.

3.4. Jollof rice

The moisture content of the jollof rice meal was 64.7 ± 2.0%, water activity of 0.942 ± 0.002, pH6.11 ± 0.26, free fatty acids 0.06% and peroxide value less than 0.01 Meq/kg. From the results, the jollof rice meal has ideal conditions to support the growth of a wide range of spoilage and pathogenic microbes unless preventative measures are taken. For example, the reported minimum pH to support the growth of *Salmonella* spp. is 3.8 although the optimum is 7–7.5; for the same organism, the minimum water activity for growth is 0.94 and the optimum 0.99.

The TBC of the meal was between lg 2.9 ± 0.83 CFU/g and within the microbiological standard for such ready meals. The general coliform count was low (lg 1.70 ± 1.94 CFU/g) and also within the standard for such ready meals. Faecal coliforms, *Salmonellae* and *Staphylococci*, were not detected. It is, however, noteworthy that isolates obtained from the general coliform plates

included *Klebsiella* spp., *Serratia marcescens* and *Salmonella* spp. The preliminary identification of *Salmonella* spp. among the general coliform colonies but not detected on the xilose lysine desoxycholate agar for *Salmonellae* count is noteworthy. Whilst this observation may infer very low levels, the fact is that very low levels of *Salmonellae* can cause illness and it is important to ensure their absence from ready meals such as jollof rice. There is a need for the use of rapid identification techniques to facilitate the examination of a wider range of colonies on all selective media.

Survival curves of *E. coli, S. aureus* and *Salmonella* spp. were studied as a function of irradiation dose. The linear regression equations obtained for the pathogens in the jollof rice meal are presented in Table 10. The results indicated that the number of surviving pathogens decreased linearly as the irradiation dose increased. The calculated D_{10} value of *E. coli* on the jollof rice meal substrate was 0.173 ± 0.01 kGy; values obtained for *S. aureus* and *Salmonella* spp. were 0.260 ± 0.04 kGy and 0.285 ± 0.04 kGy, respectively. The linear correlation coefficients of the regression lines were highly significant ($p < 0.01$), indicating a strong negative linear correlation. These results suggested that *E. coli* in jollof rice was the most sensitive to ionizing radiation compared with *S. aureus* and *Salmonella* spp. The ability of *Salmonellae* to grow at temperatures of <5°C has been reported, likewise the observation that they are less sensitive to ionizing radiation at chilling temperatures.

The relatively lower D_{10} for *E. coli* in jollof rice compared with values obtained for the poached chicken meal (0.182 kGy) and waakye meal (0.248 kGy) in this study is attributed to the different compositions of the meals. The D_{10} values for *S. aureus* were also higher in the poached chicken meal (0.271 kGy) and waakye meal (0.359 kGy). These results suggest that a relatively lower radiation dose may be required to enhance the microbiological safety of the jollof rice meal.

TABLE 10. RADIOSENSITIVITY OF PATHOGENS ON JOLLOF RICE MEAL

Pathogen	Regression equation	r^2	D_{10} value (kGy)
E. coli	$Y = -0.885X + 0.680$	0.947	0.173 ± 0.010[a]
S. aureus	$Y = -0.589X + 0.541$	0.997	0.260 ± 0.051
Salmonella spp.	$Y = -0.545X + 0.361$	0.912	0.285 ± 0.044

[a] Mean ± SD, of three independent experiments of two replicates each.

The results in Table 11 show that for the non-irradiated samples, there was an initial increase in the population of all the three pathogens, but by the 14th day, a general decrease was observed; this trend continued to day 28. For the irradiated samples, however, the population of the pathogens decreased consistently. The observed data of this report are supported by other authors. The results in Table 11 showed that 3 kGy is needed to ensure the microbiological safety of the jollof rice meal complete with gravy and beef tripe. It must be emphasized that refrigeration temperature is very critical in the storage of cook–chill meals, whether irradiated or not. It has been reported that proliferation of surviving microbes occurred rapidly at 10°C compared with 3°C at equivalent doses of irradiation. This, therefore, highlights the need for good refrigeration (0–3°C) of irradiated meals in order to achieve the maximum benefits from irradiation.

Table 12 indicates that irradiation and storage had no significant effect on the sensory quality of the gravy and beef tripe with respect to the attributes tested. The results further indicated that all the attributes of the rice were significantly affected by either the irradiation dose and/or the storage time. These observations support those reported for the poached chicken meal in this

TABLE 11. EFFECT OF IRRADIATION ON GROWTH OF PATHOGENS ON JOLLOF RICE MEAL DURING STORAGE AT 3–5°C

Pathogen	Dose (kGy)	Storage time (d)				
		0	7	14	21	28
E. coli	0	8.04 ± 0.12[a]	7.97 ± 0.23	7.65 ± 0.23	5.63 ± 0.65	5.37 ± 1.09
S. aureus	0	7.97 ± 0.69	8.41 ± 0.72	8.09 ± 0.51	7.45 ± 0.17	7.15 ± 0.16
Salmonella spp.	0	7.99 ± 0.49	8.42 ± 0.81	6.56 ± 0.31	6.79 ± 0.13	6.11 ± 0.30
E. coli	2	1.61 ± 1.41[a]	1.26 ± 1.09	0	0	0
S. aureus	2	4.02 ± 0.64	1.37 ± 1.19	1.28 ± 1.11	0	0
Salmonella spp.	2	3.56 ± 0.13	2.13 ± 0.46	1.44 ± 0.76	1.30 ± 1.12	0
E. coli	3	0	0	0	0	0
S. aureus	3	0	0	0	0	0
Salmonella spp.	3	0	0	0	0	0

[a] Mean count (lg CFU/g) of three independent experiments of two replicates each. Mean ± SD.

TABLE 12. SUMMARY OF 2-FACTOR ANALYSIS OF VARIANCE (ANOVA) WITH REPLICATION

Attribute	Dose		Storage time (d)	
	F value	P value	F value	P value
Rice:				
Colour	1.823	0.163	10.94	7E-07 (3.42, 4.13, 4.28, 6.16)
Grittiness	7.848	5E-04 (6.87, 6.41, 5.70)[a]	2.105	0.099
Hardness	13.46	2E-06 (6.77, 5.87, 5.19)	1.071	0.361
Taste	12.18	8E-06 (7.0, 6.54, 5.59)	2.935	0.033 (7.12, 6.67, 6.72, 6.90)
Aftertaste	13.59	2E-06 (6.9, 6.10, 5.83)	0.493	0.687
Gravy:				
Aroma rancidity	0.646	0.586	1.304	0.273
Aroma sharpness	1.012	0.364	0.292	0.831
Flavour sharpness	1.486	0.228	0.505	0.679
Flavour peppery	2.110	0.12	1.78	0.15
Beef tripe:				
Chewiness	0.35	0.705	0.956	0.414
Fibrousness	0.578	0.562	1.351	0.258

[a] Mean scores for 0, 1.5, 3.0 kGy, respectively, or 0, 7, 14, 21 d storage time, respectively, in brackets.

study (Section 7.2.3) in that irradiation dose and storage time had a greater impact on the sensory quality of the rice component than the chicken and gravy component. In fact, the storage time did have a highly significant effect ($p < 0.001$) on the rice (it became relatively lighter, finer and softer) as the irradiation dose increased. On the other hand the jollof rice aftertaste became more appealing and the taste improved as the irradiation dose increased (Appendix). Furthermore, the taste of jollof rice improved as the storage time

increased. These sensory data suggested that the treatment of jollof rice, gravy and beef tripe with up to 3 kGy and chilled for 21 d did not seem to have a negative impact on the sensory quality of the meal.

4. CONCLUSIONS

The pH and water activity of the prepared meals investigated were suitable for the proliferation of spoilage microorganisms, coliforms and potential pathogens.

The microbiological count of market samples of a complete waakye meal exceeded the microbiological standards for such short shelf life foods; potential pathogens were also present on the meals.

The microbiological counts on meals prepared under the HACCP plan as well as jollof rice were within the microbiological standards for such short shelf life foods. Some of the isolates of potential pathogens from waakye and its accompaniments were also present on some of the meals prepared under the HACCP. From these results, it could be concluded that although the adoption of the HACCP improved the microbiological quality of prepared meals, it did not eliminate potential pathogens. Consequently, there is a need to complement the HACCP with other processes that can eliminate pathogens in order to enhance the microbiological safety of prepared meals.

From the radiosensitivity tests, it could be concluded that complete waakye exerted protective effects on *E. coli*, *S. aureus* and *Salmonella* spp. such that their D_{10} values were higher than values obtained for the poached chicken meal and jollof rice.

From the challenge tests using *E. coli, S. aureus* and *Salmonella* spp. on the poached chicken meal or jollof rice, a 3 kGy dose was found to be sufficient for the elimination of a mixture of the potential pathogens in order to ensure the microbiological safety of the meals. In view of the relatively higher D_{10} values in complete waakye, more than a 3 kGy dose will be required to ensure the microbiological safety of this meal if all the three potential pathogens were present.

From the sensory tests, it could be concluded that vegetables such as boiled carrots are unable to withstand more than a 1 kGy dose, consequently, these should be packaged and treated separately or prepared fresh and served with the meal.

Treatment of poached chicken and gravy with 2 kGy or beef tripe and gravy in the jollof rice meal with up to 3 kGy did not have significant effects on their sensory quality. Although the 1–3 kGy doses had significant effects on the

texture but not the colour of the rice components of the poached chicken meal and jollof rice, they were acceptable to the panellists.

From the foregoing, the microbiological safety and shelf life of a poached chicken meal or jollof rice under chilled storage can be enhanced when treated with a 3 kGy dose. Such a treatment can extend the shelf life of the meals from the recommended 5 d to 28 d under chilled storage. It must be emphasized that irradiation is not a replacement for good manufacturing practices and therefore strict adherence to recommended storage temperature (0–3°C) is imperative in order to achieve maximum benefits from irradiation.

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Appendix

DESCRIPTIVE PROFILE OF PREPARED MEAL

Name: **Date:** **Dept.:** **Product:** Jollof rice

Please evaluate the intensity of the various named attributes of the jollof rice samples provided. For each attribute, judge by placing a distinct vertical line on the horizontal line given. Label each vertical line with its sample code.

437 249 572 681

Colour

Deep_____ Light

Texture (grittiness)

Fine_____Coarse

Texture (hardness)

Soft _____ Hard

Flavour (aftertaste)

Appealing _____Rancid

Taste

Nice _____ Awful

DESCRIPTIVE PROFILE OF PREPARED MEAL

Name: **Date:** **Dept.:** **Product:** Gravy

Please evaluate the intensity of the various named attributes of the gravy samples provided. For each attribute, judge by placing a distinct vertical line on the horizontal line given. Label each vertical line with its sample code.

437 249 572 681

Aroma (rancid/stale)

Fresh_____Rancid

Aroma (sharpness)

Savory _____ Pungent

Flavour (acid/sharpness)

Mild _____ Acidic

Flavour (peppery)

Mild_____ Hot

DESCRIPTIVE PROFILE OF PREPARED MEAL

Name: **Date:** **Dept.:** **Product:** Beef tripe

Please evaluate the intensity of the various named attributes of the samples of beef tripe provided. For each attribute, judge by placing a distinct vertical line on the horizontal line given. Label each vertical line with its sample code.

437 249 572 681

Texture (chewiness)

Brittle_____ Chewy

Texture (fibrousness)

Fine_____Coarse

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PART I: IMPROVEMENT OF THE MICROBIOLOGICAL SAFETY OF TWO CHILLED SEMI-PREPARED MEALS BY GAMMA IRRADIATION

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Abstract

Experimental batches of a stuffed pasta product, tortellini and slightly pre-fried breaded reconstituted turkey meat steaks with cheese and ham filling, cordon bleu, were prepared according to commercial recipes, then inoculated to 10^4 CFU/g with *Staphylococcus aureus* (in the case of tortellini) and to 10^6 CFU/g with *Listeria monocytogenes* (in the case of cordon bleu) prior to packaging in plastic pouches under a gas atmosphere of 20% CO_2 and 80% N_2. The inoculated packages were irradiated at 3 kGy (tortellini) and 2 kGy (cordon bleu) by a ^{60}Co source. The applied radiation doses were sensorially acceptable with these products. The experimental batches of tortellini were stored at 15°C, while the cordon bleu samples were stored at 5 and 9°C, respectively. Non-irradiated samples were kept together with the respective irradiated ones. Storage was continued for 4 weeks and microbiological tests were performed before and after irradiation, and subsequently after every 7 d. Besides selective estimation of the counts of the test organisms, total aerobic counts, and in the case of cordon bleu, colony counts of lactic acid bacteria, *Enterobacteriaceae*, sulphite reducing *Clostridia*, yeasts and moulds were also selectively estimated. The 3 kGy dose reduced the *S. aureus* count in tortellini below the detection limit (lg CFU = 0.26), and it remained undetectably low in the irradiated samples during the whole 28 d of storage, while the *S. aureus* count in the non-irradiated samples increased up to 10^8 CFU/g by the 8th day. The *Listeria* count in the cordon bleu was reduced by irradiation from the initial lg CFU/g = 6.1 to lg CFU/g = 3.5. At 5°C storage,

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this residual count remained stagnant up to 3–4 weeks, but started to increase at 9°C after one week. In the non-irradiated samples, the *Listeria* count increased hundredfold over 4 weeks at 5°C, and over 2 weeks at 9°C. Sulphite reducing *Clostridia* were, and remained, undetectable (lg CFU/g < 0.48) in all samples even at 9°C. The limiting factor of the shelf life of the non-irradiated poultry product was the growth of lactic acid bacteria at 9°C, whereas enhanced lipid oxidation was an unwanted side-effect of radiation treatment. The conclusion drawn from these studies is that the potential risk posed by the investigated non-spore-forming pathogenic bacteria could be considerably reduced by gamma irradiation. However, storage temperature remains a crucial factor for safety and methods should be developed to counteract the lipid oxidative effect of the radiation processing.

1. INTRODUCTION

There is a growing interest in marketing semi-prepared and prepared meals contained in modified atmosphere packages and distributed under chilled conditions instead of in the frozen state. Such products are less energy demanding and more attractive for the consumers than frozen meals. However, they are non-sterile and potential survival of some pathogenic microorganisms and/or post-processing contamination before packaging create microbiological risks and place a considerable limitation on shelf life, especially under abusive temperature conditions which may frequently occur during the retail display period and during home storage before consumption.

Irradiation is an effective way to eliminate non-spore-forming pathogens without changing the physical state of foods [1]. Therefore, the objective of the authors' work was to investigate the feasibility of diminishing the above risk by gamma irradiation of two types of chilled semi-prepared meal: a filled pasta product, tortellini, and slightly pre-fried reconstituted breaded turkey meat steaks containing cheese and ham filling (cordon bleu). For challenge tests [2], experimental batches of the tortellini and cordon bleu were inoculated with *Staphylococcus aureus* and *Listeria monocytogenes,* respectively.

2. MATERIALS AND METHODS

2.1. Test organisms and preparation of inocula

The *S. aureus* strain ATCC 6538, obtained with a code number of B01462 as a liophylized culture from the National Collection of Agricultural and Industrial Microorganisms, Budapest, was used as inoculum for tortellini.

Resuscitation of the stock culture was made in brain–heart infusion broth (Merck, 1.10493). Its growth was checked by a *Staphylococcus* enrichment broth and formation of typical colonies on Baird Parker agar (Merck, 1.07809), as well as positive catalase and coagulase tests. Storage and subculturing were done on brain heart infusion and plate count agar slopes.

For preparation of the inoculum, the bacterium was cultivated by shaking in brain–heart infusion broth at 37°C for 24 h. The cells were harvested in a refrigeration centrifuge after 15 min at rcf 27 200 (15 000 rpm), and resuspended in Sörensen's phosphate buffer of pH7.0.

Listeria monocytogenes 4ab strain No. 10, obtained from the culture collection of Dr. B. Ralovich (National Meat Research Institute, Budapest), was used as the test organism for cordon bleu. A 24 h culture produced at 30°C in brain–heart infusion broth in a shaker bath was centrifuged as above for 15 min, and the sedimented cells were resuspended in sterile water.

2.2. Test materials

A commercially prepared tortellini pasta product and cordon bleu composed of breaded and mildly pre-fried reconstituted turkey meat steaks containing a slice of ham and a slice of cheese were used as test materials. Cordon bleu is marketed presently as an aerobically packaged quick frozen food.

The tortellini dumplings were made from durum wheat flour, egg and water, and the stuffing was prepared from bread crumbs, smoked ham, cheese, salt, pepper and sodium glutamate. The pasta:filling ratio was 25:9. Water activity of the product was approximately 0.96 at 25°C, and the pH ranged between 5.5 and 6.1.

The water activity of cordon bleu was also approximately 0.96 at 25°C, and the pH of the homogenized samples was in the range of 6.24–6.30.

2.3. Inoculation and packaging of samples

Tortellini dumplings were inoculated individually to approximately 10^4 CFU/g level with 10 µL aliquots of the *S. aureus* suspension prepared as above, and the inoculated pasta (10 dumplings per pouch) were packaged by a MULTIVAC Victus packaging machine with the packaging foil of the commercial pasta producer under a gas atmosphere of approximately 20% CO_2 and 80% N_2. The residual O_2 content was 0.23%.

Individual 'steaks' of cordon bleu were inoculated with 120 µL of *L. monocytogenes* suspension, distributing it in 10 µL aliquots on the surface of the lightly pre-fried steaks, in order to obtain a *Listeria* contamination of

approximately 10^6 CFU/g of the test material. The steaks were then vacuum packed (10 bar residual pressure) into multibarrier-4 laminated foil pouches which were then refilled to 750 bar pressure with a gas mixture of approximately 20% CO_2 and 80% N_2.

2.4. Radiation processing

Numerous replicate packages of the inoculated products were irradiated by an RHγ-30 type self-shielded ^{60}Co gamma irradiator at a dose rate of 2.4 kGy/h. Processing doses were 3 kGy for tortellini and 2 kGy for cordon bleu, sensorially acceptable doses selected by preliminary experiments reported elsewhere [3]. Non-irradiated packages were kept together with the irradiated samples as controls.

2.5. Storage and microbiological testing

The experimental batches of tortellini were stored at 15°C, representing a strongly abusive but not infrequent temperature condition, while cordon bleu samples were stored at both 5 and 9°C. Storage was continued for 4 weeks and microbiological tests were performed before and after irradiation, and subsequently after every 7 d of storage.

Besides selective estimation of the counts of the test organisms, total aerobic viable cell counts, and, in the case of cordon bleu, also colony counts of lactic acid bacteria, *Enterobacteriaceae*, sulphite reducing *Clostridia*, as well as yeasts and moulds were selectively estimated from appropriate dilution levels prepared from duplicate packages per treatment.

Estimation of *S. aureus* was performed both by direct spread plating on Baird Parker agar + tellurite egg yolk emulsion (Merck, 1.03785) at 37°C (incubation for 48 h), and by *Staphylococcus* enrichment broth tubes (Merck, 1.07899), with K_2TeO_3 (Merck, 1.05164).

Selective estimation of *L. monocytogenes* was done both in Oxford agar (Merck, 1.07004) with *Listeria* supplement (Merck, 1.07006), counting after 24 and 48 h at 30°C, and by the MPN technique as well, in triplicate liquid cultures using *Listeria* enrichment broth (Merck, 1.10259) and incubation at 30°C for up to 5 d. *Listeria* positive tubes were verified by subculturing them onto Oxoid agar plates.

Total bacteria counts (TBCs) were estimated in casein-pepton-dextrose-yeast agar (Merck, 1.05463) at 30°C with incubation for 2–3 d.

The counts of bacterial spores were determined by plating as the total aerobic counts after 10 min heat treatment at 80°C of the first dilution level.

Lactic acid bacteria were counted by plating into MRS medium (Merck 1.10661) with 1.5% agar, covered after inoculation with a surface layer of the same medium, and incubated at 30°C for 3 d.

Enterobacteriaceae counts were estimated by plating in violet red bile glucose agar (Oxoid, CM485), overlayered with the same medium, and incubated at 37°C for 24 h.

Sulphite reducing clostridia were investigated by plating into differential clostridial agar according to Wenk (Merck, 1.10259) supplemented with ferric ammonium citrate (1 g/L) and Na_2SO_3 (0.75 g/L), incubated in Oxoid HP11 anaerobic jars containing Oxoid Anaerogen AN35 oxygen adsorbent.

Yeasts and moulds were estimated in thin layers of DRBC agar (Oxoid CM 727) with chloramphenicol and supplement (Oxoid SR 078E), incubated for 6 d at room temperature.

2.6. Measurement of head space gas composition

The head space of the modified atmosphere packaged samples was checked by a SERVOMEX infrared gas analyser PA 404 for CO_2 and by a SERVOMEX oxygen analyser 574 for O_2 content.

2.7. Measurement of pH and water activity

The pH was measured by a Physitemp electric pH meter and water activity was estimated by the crystal liquefaction method.

2.8. Thiamine content

Thiamine (vitamin B_1) content was estimated by a microbiological method [4]. This specific and sensitive method is based on the extent of growth of a *Lactobacillus* test organism, which has a requirement for thiamine, on a thiamine-free basal medium when aliquots of extracts of a thiamine containing food are added to it. The growth response is measured turbidimetrically after an incubation period at 30°C and the optical density is compared to a standard curve.

2.9. Lipid oxidation

Lipid oxidation was investigated by determination of thiobarbituric acid reactive substances (TBARS values) according to Newburg and Concon [5], expressed in malonaldehyde concentrations. Malonaldehyde is an oxidative breakdown product formed mainly from peroxidized polyunsaturated fatty acids.

3. RESULTS

3.1. Radiation processing of modified atmosphere package chilled tortellini

3.1.1. Microbiological effects

Results of the microbiological tests are summarized in Table 1.

The initial total aerobic viable cell counts of the inoculated samples were determined by the *S. aureus* inoculum, because the original total aerobic cell count of the non-irradiated samples was 2 log cycles less, approximately lg CFU = 2.3/g, composed mainly of aerobic bacterial spores.

As a result of irradiation, the *S. aureus* count was reduced to below the detection limit (which was lower in the case of the MPN technique), i.e. less than lg CFU = 0.26. Thus, the radiation treatment resulted in a more than 3 log cycle reduction of the test organism in the pasta product.

Considering the initial TBCs of the irradiated samples at the beginning of the storage (lg CFU ≈ 1.5/g), the aerobic spores of the 'native' microbiota was reduced by somewhat less than one log cycle by the 3 kGy dose. The surviving spore formers were unable to grow in the irradiated samples under the experimental conditions.

The *S. aureus* inoculum grew up to the 10^8 CFU/g level in the non-irradiated samples within 8 d, although no *S. aureus* cells were found in the irradiated batch during 28 d storage at 15°C.

TABLE 1. LOGARITHM OF VIABLE CELL COUNTS IN DUPLICATE SAMPLES OF TORTELLINI STORED AT 15°C

Viable cell counts	Storage time (d)							
	0		8		14		28	
	0 kGy	3 kGy	0 kGy	3 kGy	0 kGy	3 kGy	0 kGy	3 kGy
TBCs	4.32	1.48	7.99	4.52	8.11	4.81	n.i.[a]	5.75
	4.28	1.54	7.91	4.18	8.04	4.70	n.i.	7.43
S. aureus (Baird Parker)	4.08	<1.70	7.98	<1.70	8.04	<1.70	n.i.	<1.70
	4.04	<1.70	7.95	<1.70	8.04	<1.70	n.i.	<1.70
S. aureus (enriched broth)	4.08	<0.26	7.88	<0.26	7.36	<0.26	n.i.	<0.26
	4.67	<0.26	8.36	<0.26	7.36	<0.26	n.i.	<0.26

[a] n.i. = not investigated.

The aerobic total bacteria cell counts started to increase in the irradiated samples only after 8 d storage, and their slow increase afterwards was due to some yeasts and micrococci forming white colonies distinctly different from those of the test organism.

3.1.2. Thiamine content

The thiamine content of the untreated samples was found to be 34 μg/100 g, with an accuracy of +/–10%, and it was not changed by the radiation dose applied.

3.2. Radiation processing of the modified atmosphere packaged chilled cordon bleu

3.2.1. Microbiological effects

In order to determine how much microbial loads have been carried by the pre-fried cordon bleu, individual components of uninoculated and non-irradiated steaks were investigated for TBCs, bacterial spores (cells surviving at 80°C for 10 min) and viable cell counts of lactobacilli, at the beginning of the storage experiment. The results are shown in Table 2.

These results show that, although in relatively low numbers, not only bacterial spores but also vegetative bacterial cells can be recovered from each component of the product. Therefore, in principle, there is an opportunity that eventual pathogenic contamination might occur in such product.

TABLE 2. MICROBIAL LOAD OF INDIVIDUAL COMPONENTS OF THE UNINOCULATED AND NON-IRRADIATED CORDON BLEU (lg CFU/g VALUES OF DUPLICATE SAMPLES)

Component	TBC	Spore count	Lactobacilli
Crumb	2.30	0.95	<0.48
	1.84	0.78	<0.48
Turkey meat	2.15	0.48	1.48
	2.73	0.48	1.89
Ham	1.32	1.48	<0.48
	1.71	<0.48	<0.48
Cheese	2.36	1.89	0.78
	2.32	1.82	0.78

TABLE 3. MICROBIOLOGICAL CHANGES OF INOCULATED NON-IRRADIATED AND INOCULATED IRRADIATED (2 kGy) MODIFIED ATMOSPHERE PACKAGED CORDON BLEU AT 5°C STORAGE TEMPERATURE (lg CFU/g VALUES OF DUPLICATE SAMPLES)

Parameter	Untreated (d)					Irradiated (d)				
	1	7	14	21	28	1	7	14	21	28
Listeria counts	6.08 6.15	6.18 6.15	6.73 6.66	7.48 7.28	8.08 7.56	3.53 3.57	3.30 3.26	2.92 2.89	2.62 2.68	5.15 3.52
Lactobacilli	<0.48 <0.48	<0.48 <0.48	1.26 <0.48	4.00 6.11	6.41 6.23	<0.48 <0.48	<0.48 <0.48	<0.48 <0.48	<0.48 <0.48	<0.48 <0.48
pH	6.17 6.16	6.14 6.12	6.02 6.04	6.02 6.00	5.73 5.87	6.15 6.14	6.14 6.13	6.03 6.04	6.08 6.07	6.03 6.02

TABLE 4. MICROBIOLOGICAL CHANGES OF INOCULATED NON-IRRADIATED AND INOCULATED IRRADIATED (2 kGy) MODIFIED ATMOSPHERE PACKAGED CORDON BLEU AT 9°C STORAGE TEMPERATURE (lg CFU/g VALUES OF DUPLICATE SAMPLES)

Parameter	Untreated (d)					Irradiated (d)				
	1	7	14	21	28	1	7	14	21	28
Listeria counts	6.08 6.15	6.98 7.43	8.15 8.04	8.90 8.46	8.51 8.41	3.53 3.57	3.74 3.46	6.52 6.46	7.71 7.79	8.11 8.15
Lactobacilli	<0.48 <0.48	<0.48 <0.48	1.62 <0.48	4.15 6.36	7.41 8.04	<0.48 <0.48	<0.48 <0.48	<0.48 <0.48	<0.48 <0.48	<0.48 <0.48
pH	6.17 6.16	6.07 6.06	5.74 5.72	5.74 5.63	5.56 5.58	6.15 6.14	6.13 6.14	6.04 6.05	5.94 5.92	5.72 5.75

The *Listeria* counts and the counts of lactobacilli of the inoculated samples are shown together with the pH values in Tables 3 and 4 as a function of irradiation and storage time.

Owing to the heavy inoculation and the low number of the native microbiota, the total viable cell counts are not shown, because they were equal with the *Listeria* counts. Other types of microorganism (spore counts, *Enterobacteriaceae*, yeasts and moulds) selectively investigated were and remained under the detection level of lg CFU/g = 0.48.

The *Listeria* count in cordon bleu was reduced by irradiation from the initial lg CFU/g = 6.1 to lg CFU/g = 3.5. During storage at 5°C (Table 3), this residual count remained stagnant up to 3–4 weeks, but at 9°C (Table 4) it started to increase after the first week. In the non-irradiated samples, the *Listeria* counts increased hundredfold over the 4 weeks at 5°C, and over the first 2 weeks at 9°C. The limiting factor of the microbiological shelf life of the non-irradiated product was the growth of lactic acid bacteria at 9°C, although they were in undetectably low numbers during the first two weeks of storage. They apparently survived the pre-frying process heterogeneously distributed and in very low counts inside the product.

The radiation treatment was effective in eliminating this low number of lactic acid bacteria. In spite of the buffer capacity of the samples, the decrease in the pH of the non-irradiated samples reflected the growth of lactic acid bacteria. However, a slight decrease of the pH was also noted in the irradiated samples during the course of storage, when an extensive growth of the surviving *Listeria* was observed.

3.2.2. Gas composition of the head space of the experimental product

The CO_2 and O_2 contents of the head space of the packages as a function of the storage time and temperature are given in Table 5.

TABLE 5. THE CO_2 AND O_2 CONTENTS OF THE HEAD SPACE OF MODIFIED ATMOSPHERE PACKAGED CORDON BLEU AS A FUNCTION OF STORAGE TIME AND TEMPERATURE (MEASUREMENTS OF DUPLICATE PACKAGES)

Treatment	Temperature (°C)	CO_2 (%)						O_2 (%)					
		Initial	1 d	7 d	14 d	21 d	28 d	Initial	1 d	7 d	14 d	21 d	28 d
0 kGy	5	19	10	12	10	12	12	1.4	2.0	2.0	1.6	1.8	1.9
				12	11	8	12			1.8	1.5	1.8	1.8
	9	20	12	14	10	20	18	1.4	1.8	1.7	1.5	1.7	1.7
				12	11	17	18			1.4	1.4	1.7	1.7
2 kGy	5	20	11	9	11	10	10	1.5	1.7	1.8	1.3	2.0	2.0
				12	12	11	11			1.7	1.0	2.0	2.0
	9	18	11	12	15	14	16	1.4	1.7	1.3	0.8	1.9	2.0
				10	12	13	14			1.7	0.4	1.9	1.9

These measurements revealed that a considerable part of the CO_2 introduced during packaging was soon dissolved in the high moisture product. Thus, the equilibrium CO_2 concentration in the head space became 10–12% in those packages which have been stored at 5°C, while at 9°C the head space concentration of CO_2 increased again when bacterial growth became intense. The O_2 contents were most frequently between 1.5 and 2.0%, irrespective of the storage temperature and time.

3.2.3. Appearance of the samples and lipid oxidation

The radiation treatment did not change the appearance of the samples and no 'off' odour was noted at the opening of the pouches in the first half of the storage period. However, after 2 weeks of refrigerated storage, the breadcrumb coating of the steaks started to develop a less freshly fried, moist appearance, independent of the radiation treatment and the cheese slice inside the non-irradiated samples started to liquefy, more intensely at 9°C than at 5°C. Over the last two weeks of storage, the samples gradually lost their fresh, attractive appearance and produced some oily exudation. The untreated samples developed a stale odour, while in the irradiated samples a rancid odour was noted when they were prepared with an homogenizer for microbiological testing.

The TBARS values of cordon bleu samples measured directly after irradiation and after 4 weeks storage are given in Table 6. The increased TBAR values showed that lipid oxidation progressed during storage and it was enhanced by the radiation treatment.

TABLE 6. TBARS VALUES OF CORDON BLEU SAMPLES AS A FUNCTION OF IRRADIATION AND STORAGE

Radiation dose (kGy)	TBARS (malonaldehyde mg/kg)					
	Directly after irradiation		After 4 weeks storage at 5°C		9°C	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	0.38	0.04	0.85	0.03	1.00	0.24
2	0.51	0.03	1.58	0.20	1.86	0.25

4. DISCUSSION

The international literature on filled pasta products shows that the incidence of contamination with *S. aureus* is not infrequent [6–10]. The water activity level of the product investigated in the authors' experiments would not exclude either the opportunity for growth and enterotoxin formation of *S. aureus* if the pathogen would contaminate this type of product. Owing to the high heat resistance of *Staphylococcus* enterotoxins, the final cooking of these stuffed pastas cannot inactivate pre-formed toxins. Therefore, inoculated pack studies were undertaken. The authors' experiments showed that *S. aureus* was growing readily at 15°C in the inoculated tortellini (water activity = 0.96), but the 10^4/g artificial contamination level of the pathogen could be eliminated from the experimental samples by 3 kGy of gamma radiation, a sensorially acceptable dose level, which did not decrease the thiamine content of the product.

The present commercial production technology cannot fully ensure the safety of the poultry product cordon bleu for non-frozen storage even under modified atmosphere packaging, because even some vegetative bacteria might survive the mild pre-frying of the product. Survival and growth of lactic acid bacteria in the non-irradiated experimental batches make it probable that *L. monocytogenes*, a ubiquitous environmental contaminant, might eventually be present too, and this is able to multiply during refrigerated storage. The *Listeria* count in cordon bleu was reduced by 2.6 lg units by the sensorially acceptable 2 kGy gamma radiation dose. This extent of lethality is close to the result of Thayer et al. [11] who found that the D-value of *L. monocytogenes* was 0.63 ± 0.06 kGy in ground cooked turkey meat. The observed reduction in *L. monocytogenes*, the most radiation resistant non-spore former [12], could result in a *Listeria*-free product, if the contamination level of this pathogen is not higher than 10^2 CFU/g. In the authors' heavily contaminated experimental batch, the surviving level of the test organism remained stagnant at 5°C for up to 3–4 weeks in the irradiated samples, while the *Listeria* counts of the non-irradiated samples increased a hundredfold over 4 weeks at 5°C, and over 2 weeks at 9°C. In irradiated samples stored at 9°C, the surviving *Listeria* also started to grow after one week. This slow recovery of the radiation survivors is in agreement with the observation of Patterson et al. [13] who irradiated raw and cooked poultry meat inoculated with *L. monocytogenes* with doses of 1.0 and 2.5 kGy and found that irradiation resulted in significantly increased lag times for this pathogen. Regarding psychrotrophic spore forming pathogens, it is reassuring that sulphite reducing *Clostridia* were, and remained, undetectable during the entire period of the experiments, even in the non-irradiated samples. The results can be compared with those of Hashim et al. [14] who

irradiated chicken meat with doses of 1.66–2.86 kGy to determine the effects of radiation processing on the sensory attributes of both raw and cooked meat, but did not find significant effects upon the appearance or taste of cooked breast meat. Kanatt et al. [15] irradiated minced chicken with 2.5 kGy and stored it at 0–3°C for up to 4 weeks. The irradiated meat was microbiologically safe and sensorially acceptable in the unfrozen state up to the end of this storage; the non-irradiated minced chicken had a shelf life of less than 2 weeks.

5. CONCLUSIONS

One can conclude from these studies that the potential microbiological risk in the experimental products posed by the investigated non-spore forming pathogenic bacteria could be considerably reduced by sensorially acceptable radiation doses. However, storage temperature remains a crucial factor of safety, and increased lipid oxidation is a limiting factor of the shelf life of the irradiated poultry product even under the low O_2 concentration of modified atmosphere packaging. The possibility of counteracting lipid oxidation by efficient use of antioxidative additives will be the subject of further studies.

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PART II: EFFECTS OF GAMMA IRRADIATION ON LIPID AND CHOLESTEROL OXIDATION IN MECHANICALLY DEBONED TURKEY MEAT

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Abstract

The pasteurizing effect of a 2 kGy radiation dose on non-frozen mechanically deboned turkey meat was achieved without increase in cholesterol oxidation products or increases in thiobarbituric acid reactive substance values during 15 d of chilled storage following the treatments, while untreated samples were spoiled. The addition of antioxidants, such as thyme oil or α-tocopherol plus ascorbic acid, significantly inhibited the oxidative changes of cholesterol and lipids during 3 kGy treatment.

1. INTRODUCTION

The authors' earlier work (see Part I and Refs [1, 2]), and other work in the literature [3–7], showed that gamma irradiation accelerates the formation of cholesterol oxidation products (COPs) and increases the thiobarbituric acid reactive substance (TBARS) values. All of these studies showed that the presence of oxygen, which depends on packaging and treatment conditions, is one of the most important factors in the formation of COPs and lipid oxidation. Certain cholesterol oxidation compounds have potentially undesirable biological activities such as atherogenicity, atherosclerosis, cytotoxicity, mutagenicity and carcinogenicity, and furthermore may cause cell membrane

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damage as well as inhibiting cholesterol biosynthesis. Current knowledge of cholesterol oxidation and its biological effects has been reviewed in detail by Guardiola et al. [8]. Cholesterol oxides are present in certain foodstuffs in concentrations of a few milligrams per kilogram. Cholesterol and lipid oxidation are affected by various factors, such as light, heat, radiation, oxygen, moisture, pH, certain pro-oxidizing agents, processing procedures and storage conditions. In the authors' studies, the effects of gamma irradiation on the microbiological status, as well as on the oxidation of cholesterol and other lipids, were compared. The efficiency of natural antioxidants in suppressing the undesirable oxidative changes during the treatments in fresh and chill stored samples was also studied.

2. MATERIALS AND METHODS

2.1. Test material and packaging

Numerous portions of 25–40 g of commercial, mechanically deboned turkey meat were packaged into small plastic pouches formed from multi-barrier 4 PE-PA laminated foil of 100 μm thickness (produced by WIPAK, Finland), oxygen transmittance: 5 $mL \cdot m^{-2} \cdot 24\ h^{-1}$atm at 23°C and 50% relative humidity, and sealed without evacuation by a MULTIVAC model 300 machine (Haggenmüller, Walfertschwerden, Germany).

2.2. Ionizing radiation

The MDM samples were treated with dosage levels of 2 and 3 kGy in a ^{60}Co self-shielded radiation source, type RH-γ-30, at a dose rate of 3.9 kGy/h at approximately 5°C in the presence of antioxidant additives, then stored together with non-irradiated samples at 4°C for 15 d.

2.3. Addition of antioxidants

For assessing the effects of antioxidants, before packaging and radiation treatment, the mechanically deboned turkey meat was divided into three experimental batches: (1) without antioxidants, (2) with addition of 250 mg thyme oil/100 g and (3) with addition of 12 mg α-tocopherol plus 25 mg ascorbic acid/100 g sample.

2.4. Analyses

2.4.1. Microbiological status

Total bacteria counts (TBCs) in tryptone glucose extract (TGE) agar plates (Merck 1.05463) and *Enterobacteriaceae* counts on violet red bile glucose (VRBG) agar (Oxoid CM485) were estimated after incubation at 30°C for 48 h.

2.4.2. Measurement of thiobarbituric acid reactive substances (TBARS values)

The TBARS values were estimated according to Ramanathan and Das [9] and expressed as malondialdehyde.

2.4.3. Determination of cholesterol oxides

Approximately 50 g samples of meat or 100 g of chicken liver were extracted with chloroform/methanol (2:1, v) according to Folch et al. [10]. After evaporation of solvents, the lipid extract was dissolved in 200 mL 1M potassium hydroxide in methanol and saponified by refluxing for 60 min under a stream of nitrogen. A 200 mL volume of distilled water was added to the saponified mixture and the non-saponified organic layer was extracted three times with 100 mL of di-isopropyl-ether (Fluka AG, puriss) and the combined fractions were washed three times with 200 mL distilled water and dried with anhydrous sodium sulphate [11]. The filtrate was evaporated to dryness under vacuum. The residue was dissolved in chloroform/methanol (2:1, v) to make a concentration of 0.1 g/mL [1].

2.4.4. Separation and identification of cholesterol oxidation derivatives

The non-saponifiable extract was analysed by HPTLC using pre-coated HPTLC Sil G 60F254 thin layer 10 cm × 10 cm glass plates, purchased from E. Merck (Darmstadt, Germany). The chromato plates were developed twice in heptane-ethylacetate (1:1, v). The plates were examined under UV light (254 nm) and sprayed with 100 g/L of $CuSO_4 \cdot 5H_2O$ dissolved in 85 mL/L phosporic acid and heated to 110°C for several minutes for colour development. The identity of spots was confirmed by comparison with authentic standards of known colour and R_f values [1].

2.4.5. Quantitation

The spots of silica gel adsorbents containing oxysterols were scraped and eluted. The determination of oxycholesterols was performed by the test system cholesterol enzymatique PAP 250 ref 61225 which is based on the effect of cholesterol oxidase [12].

3. RESULTS

3.1. Effects of 2 kGy irradiation and 15 d chilled storage

The effects of irradiation and chilled storage on the total aerobic bacteria cell count are shown in Table 1.

The radiation treatment resulted in a one log cycle reduction of the viable cell count and no further changes were observed during 15 d storage of treated samples, while the untreated samples were spoiled.

3.1.1. Sensory changes

The radiation treatment did not generate an off odour or colour changes.

3.1.2. Cholesterol oxidation

Table 2 presents the results of COPs measurements in untreated and irradiated samples, respectively.

TABLE 1. TOTAL BACTERIA COUNTS IN MECHANICALLY DEBONED TURKEY MEAT (lg CFU/g) AFTER 2 kGy TREATMENT AND 15 d CHILLED STORAGE

	Chilled storage (+4°C)	
	0 d	15 d
Control	7×10^3	8×10^{7a}
2 kGy	8×10^2	7×10^2

[a] The samples were spoiled.

TABLE 2. CONCENTRATION OF TOTAL COPs (mg/kg) IN UNTREATED SAMPLES OF MECHANICALLY DEBONED TURKEY MEAT AND THOSE TREATED WITH 2 kGy, FOLLOWED BY REFRIGERATED STORAGE FOR 15 d

	Control		2 kGy	
	0 d	15 d	0 d	15 d
Total COPs	0.18	Samples spoiled	0.19	0.67

There were measurable initial levels of total COPs in the non-irradiated material, although no significant increase was noted directly after the relatively low dose. After 15 d storage, a measurable increase of COPS was observed in the non-spoiled, irradiated samples. The low initial TBARs value (0.035 malonaldehyde eqv·mg/kg) remained unchanged.

3.2. Effect of natural antioxidants on the microbial status and undesirable oxidative changes in 3 kGy irradiated mechanically deboned turkey meat stored chilled for 15 d

The aim of this experiment was to reduce the undesirable oxidative changes during the 3 kGy irradiation and refrigerated storage.

3.2.1. Microbiological status

The 3 kGy radiation treatment reduced the TBC by 3.5 lg units (from lg CFU/g = 4.75 to lg CFU/g = 1.20) and the *Enterobacteriaceae* count from lg CFU/g = 4.1 to below the detection limit (lg CFU/g < 1.0) as estimated directly after treatment (0 d) in the antioxidant free samples. In the thyme oil containing samples, the residual TBC at 0 d was lg CFU/g = 1.6 whereas it was lg CFU/g = 2.0 in the tocopherol ascorbic acid containing samples. After 15 d of chilled storage, non-irradiated samples reached a spoilage level of TBCs (lg CFU/g = 8.75).

3.2.2. Cholesterol oxidation and TBARS

Results of COPs and TBARS measurements are presented in Tables 3 and 4, respectively.

It can be seen from Table 3 that several COPs were found even in the non-irradiated samples, before irradiation and storage. Gamma irradiation enhanced the cholesterol and lipid oxidation (causing an approximately 20%

TABLE 3. CONCENTRATION OF COPs (mg/kg) IN NON-IRRADIATED AND GAMMA IRRADIATED MECHANICALLY DEBONED TURKEY MEAT[a] (AVERAGE OF DUPLICATED SAMPLES IN TWO PARALLEL EXPERIMENTS)

COPs	Day 0 (non-irradiated)			Day 0 (3 kGy)			Day 15 at 4°C (3 kGy)		
	C[b]	AO[c]	T[d]	C	AO	T	C	AO	T
7α-OH	2.04	1.11	0.89	3.06	1.40	1.34	3.63	1.73	1.78
7β-OH	2.12	1.04	0.79	2.02	1.58	0.92	2.14	1.64	2.54
7-keto	2.50	n.d.[e]	n.d.	2.70	n.d.	n.d.	2.39	1.67	0.97
5α,6α-epoxide	2.30	n.d.	n.d.	2.28	n.d.	n.d.	2.37	n.d.	n.d.
Total	8.96	2.15	1.68	10.06	2.98	2.26	10.53	5.04	5.29

[a] Cholesterol content: 668.6 mg/kg.
[b] C: antioxidant-free control.
[c] AO: α-tocopherol plus ascorbic acid.
[d] T: thyme oil.
[e] n.d.: <0.3 mg/kg; 7α-OH: 7α-hydroxycholesterol; 7β-OH: 7β-hydroxycholesterol; 7-keto: 7-ketocholesterol; 5α,6α-epoxide: cholesterol-5α,6α epoxide.

TABLE 4. TBARS (malonaldehyde mg/kg) IN NON-IRRADIATED AND GAMMA IRRADIATED MECHANICALLY DEBONED TURKEY MEAT

Day 0 (non-irradiated)			Day 0 (3 kGy)			Day 15 at 4°C (3 kGy)		
C[a]	AO[b]	T[c]	C	AO	T	C	AO	T
4.42	1.64	1.35	7.43	2.31	1.61	5.35	2.47	2.06

[a] C: antioxidant-free control.
[b] AO: α-tocopherol plus ascorbic acid.
[c] T: thyme oil.

increase in the COPs level and a 50% increase in TBARS values) at 0 d in the antioxidant free samples. The presence of antioxidants reduced significantly both the COPs and TBARS values (Table 4).

The addition of thyme oil caused favourable changes in colour, the slightly brownish colour becoming the pink–red characteristic of fresh products. The antioxidants in this experiment were highly effective in preventing oxidative changes.

4. DISCUSSION AND CONCLUSION

Turkey meat with a low endogenous level of tocopherols is rather susceptible to oxidation of cholesterol and other lipids. Irradiation increased the microbiological stability during chilled storage without sensory changes. The pasteurizing 3 kGy dose enhanced, under aerobic storage conditions, the concentration of COPs and TBARs. The COPs formed in the MDM samples were mainly 7α-hydroxycholesterol, 7β-hydroxycholesterol (early and sensitive indicators of oxidation of cholesterol), 7-ketocholesterol and cholesterol-5α, 6α-epoxide. These observations confirmed literature data [13]. The antioxidants were highly effective in preventing oxidative changes. The mechanism behind the decrease in the concentrations of preformed oxidation products due to the addition of antioxidants is not yet clear and will be investigated. The reduction/destruction of the intermediate peroxides might be involved in this process.

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PART III: COMPARING OBSERVED GROWTH OF SELECTED TEST ORGANISMS IN FOOD IRRADIATION STUDIES WITH GROWTH PREDICTIONS CALCULATED BY COMBASE SOFTWARES

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Abstract

As a result of intensive predictive microbiological modelling activities, several computer programs and softwares became available recently for facilitating microbiological risk assessment. Among these tools, the establishment of the ComBase, an international database and its predictive modelling softwares of the Pathogen Modelling Program (PMP) set up by the USDA Eastern Regional Research Center, Wyndmore, PA, and the Food Micromodel/Growth Predictor by the United Kingdom's Institute of Food Research, Norwich, are most important. The authors have used the PMP 6.1 software version of ComBase as a preliminary trial to compare observed growth of selected test organisms in relation to their food irradiation work during recent years within the FAO/IAEA Coordinated Food Irradiation Research Projects (D6.10.23 and D6.20.07) with the predicted growth on the basis of growth models available in ComBase for the same species as those of the authors' test organisms. The results of challenge tests with *Listeria monocytogenes* inoculum in untreated or irradiated experimental batches of semi-prepared breaded turkey meat steaks (cordon bleu), sliced tomato, sliced watermelon, sliced cantaloupe and sous vide processed mixed vegetables, as well as *Staphylococcus aureus* inoculum of a pasta product, tortellini, were compared with their respective growth models under relevant environmental conditions. This comparison showed good fits in the case of non-irradiated and high moisture food samples, but growth of radiation survivors lagged behind the predicted values.

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1. INTRODUCTION

As a result of intensive predictive microbiological modelling activities, several computer programs and softwares recently became available for facilitating microbiological risk assessment. Among these tools, the establishment of ComBase, an international database and computer program, is the most important. The authors obtained permission to use it now for teaching and research purposes. ComBase is the result of extensive cooperative efforts and it combines information and predictive modelling softwares of the Pathogen Modelling Program (PMP) set up by the USDA Eastern Regional Research Centre, Wyndmore, PA, and the Food Micromodel/Growth Predictor program created by the United Kingdom's Institute of Food Research, Norwich [1].

2. METHODS

Regarding growth prediction of pathogenic bacteria, the authors have used the PMP 6.1 software version in a preliminary trial to compare observed growth of selected test organisms in relation to their food irradiation work within the FAO/IAEA Coordinated Food Irradiation Research Projects D6.10.23 (Testing the efficiency and uncertainty of sample processing for analysis of food contaminants) and D6.20.07 (Irradiation to ensure the safety and quality of prepared meals) in recent years with the predicted growth provided by growth models available for the same species of test organisms.

Figure 1 shows one example of the screen of PMP 6.1, displaying query panels for selection of model and input factors (culture conditions) as well as output panels illustrating predicted data points, lag phase duration, generation time and a graphical representation of the prediction.

3. RESULTS AND DISCUSSION

Figures 2–4 show microbiological ecological conditions and observed growth data from the authors' experiments in comparison with predicted values of the PMP software for reasonably similar or identical microbiological ecological conditions. It should be emphasised, however, that the predictive models of PMP are based only on output from laboratory experiments with complex laboratory media. Thus, the PMP represents a sort of worst case scenario, which does not necessarily exist in specific foods, therefore, its data are products of safe (conservative) predictions.

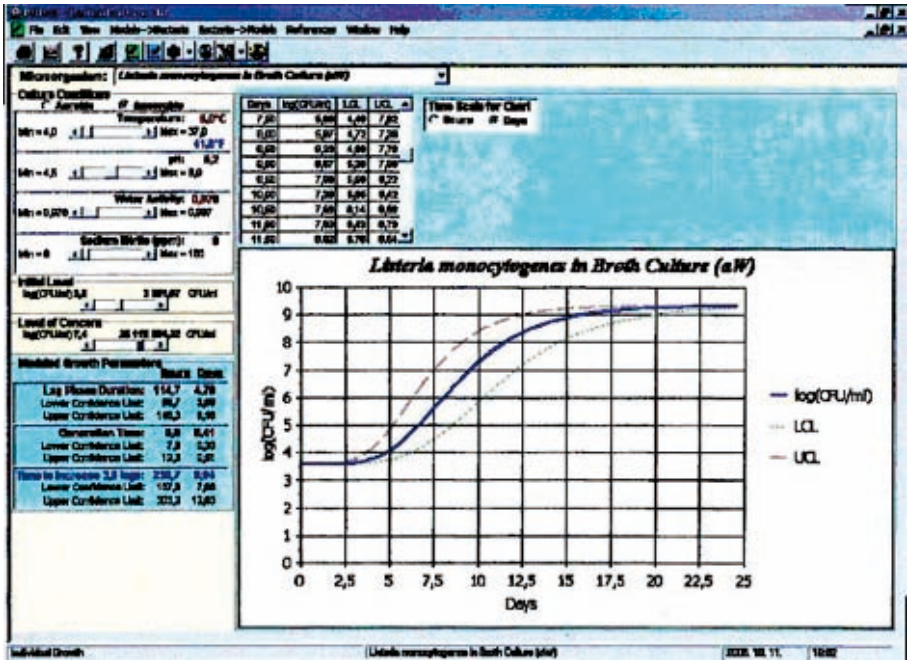

FIG. 1. Screen of the PMP 6.1 software.

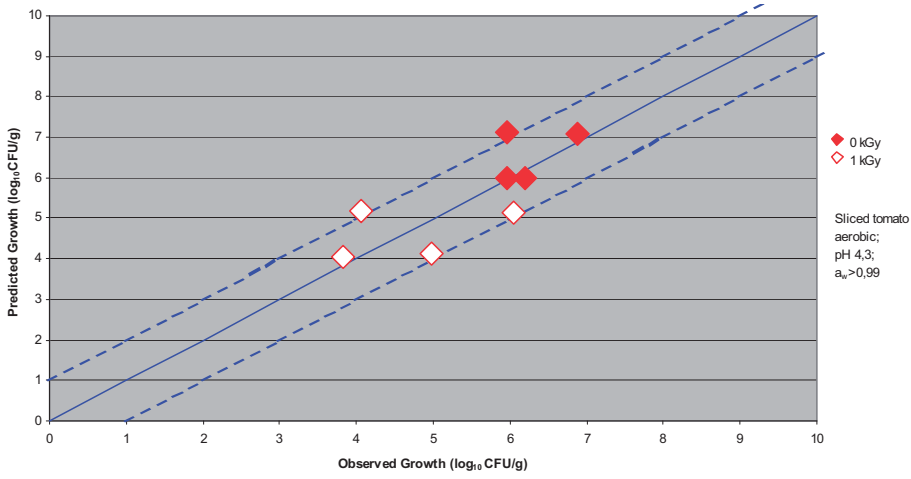

FIG. 2. Relation of observed and predicted growth of Listeria monocytogenes *in untreated and irradiated samples of sliced tomato.*

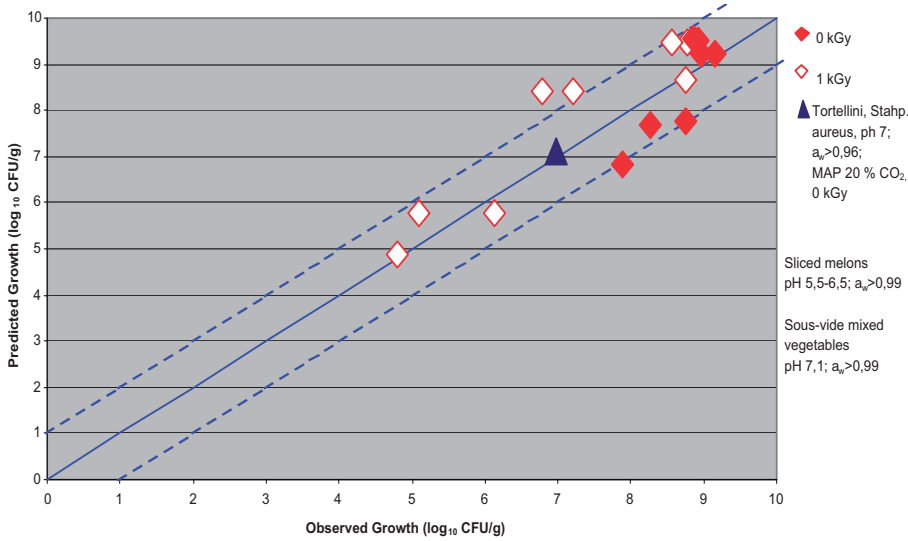

FIG. 3. Relation of observed and predicted growth of L. monocytogenes *in untreated and irradiated samples of sliced melon and sous vide mixed vegetables.*

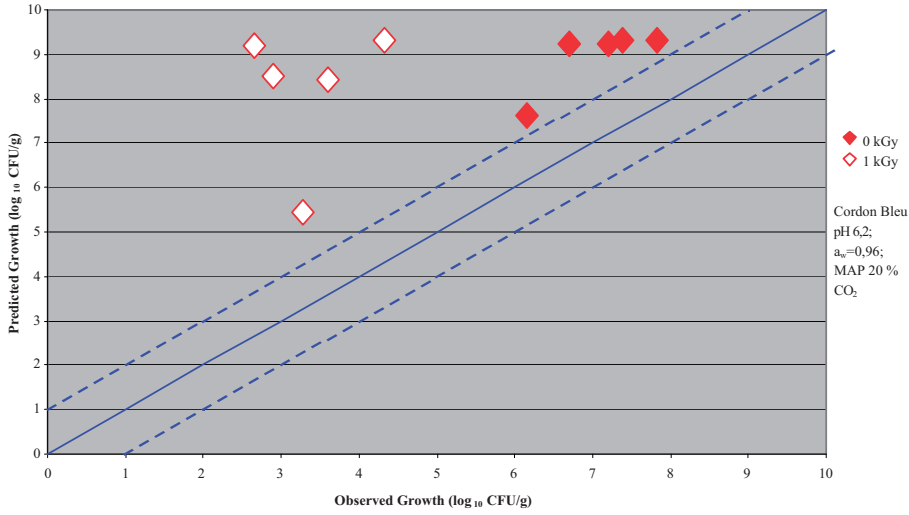

FIG. 4. Relation of observed and predicted growth of L. monocytogenes *in untreated and irradiated samples of cordon bleu.*

For *L. monocytogenes* growth predictions, the source of the predictive model is the work of Buchanan et al. [2]. They applied triptose phosphate broth for sound *L. monocytognes Scott A* inocula, the growth data were analysed by regression analysis to generate best fit Gompertz equations [3], and developed a multivariant response surface model for predicting the growth. For *Staphylococcus aureus* growth predictions, the source of data is Buchanan et al. [4], who generated growth curves by fitting the data obtained for *S. aureus 196E* in brain heart infusion broth, and quadratic response surface models for growth kinetics.

4. CONCLUSIONS

Evaluation of the information illustrated in Figs 2–4 allows the following conclusions to be drawn.

In high water activity sliced vegetable products such as sliced tomato, sliced water melon and cantaloupe as well as in the sous-vide mixed vegetables, the *L. monocytogenes* propagated at least as well as the PMP culture, thus the observed and predicted values were close to or in overlapping ranges of each other.

The *S. aureus* in the filled pasta (tortellini) also grew as well in this modified atmosphere packaged product as in the anaerobic PMP culture, resulting in a validated prediction.

Regarding cordon bleu inoculated with the authors' *L. monocytogenes* strain, the predictive counts are always higher (as for the authors' irradiated samples, they are much higher) than the observed counts. The overall difference may be due to the fact that the PMP data are based on anaerobic conditions, whereas the authors' challenge tests have been performed under modified atmosphere packaged conditions, and the increased CO_2 concentration in the packaged samples was probably affecting growth of *L. monocytogenes* more than the simple anoxic condition of the model. In addition, in the food system, competition from the native microbiota might result in less growth than in the pure culture of the PMP experiments.

Comparing the differences between predicted and observed growth in the case of radiation survivors in food samples, the Δ values were always considerably higher than those observed with non-irradiated samples, which probably means that the surviving populations in the irradiated foods, owing to their radiation injury, had longer lag phases, i.e. had less ability to start regrowth than non-irradiated populations under the same environmental conditions (see also Ref. [5]).

The authors' initial experience with the computer modelling of the growth of specific foodborne pathogens is reassuring. Therefore, further work with additional ComBase softwares would be worthwhile, offering an efficient tool for risk estimation for selected marketing scenarios. It is planned to use, in addition to the latest version of PMP, the Growth Predictor software, which is based on a dynamic growth model developed by Baranyi et al. [6, 7], since it is promising to understand and recognize better the role of physiological status of the critical organisms and thereby their lag time (i.e. no-growth) periods being of crucial importance to the safety or shelf life of the prepared and processed foods.

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RADIATION PROCESSING TO ENSURE THE SAFETY AND QUALITY OF ETHNIC PREPARED MEALS

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Abstract

The effect of gamma radiation on microbiological, chemical and sensory qualities of some Indian ethnic dishes, including items from a breakfast menu, meal components and complete meals, as well as some commercially available traditional Indian ready-to-eat meat products, were investigated. Initial total bacteria counts (TBCs) were in the range of 1–3 lg CFU/g in the case of samples prepared in the laboratory, while the counts were higher (3.5–5 lg CFU/g) in the commercial meat products. The TBC increased rapidly during storage at 0–3°C in non-irradiated samples. Radiation processing resulted in a dose dependent reduction in the total bacterial counts. *Staphylococcus* spp. was completely eliminated by irradiation (1–2 kGy). A dose of 3 kGy was found to be optimal for extending the shelf life of the commercial products by more than 2 weeks at 0–3°C compared with the corresponding non-irradiated controls. Lipid peroxidation monitored in terms of thiobarbituric acid reactive substances content increased marginally on irradiation and with storage. However, the sensory attributes of products were not significantly affected. The safety of irradiated chilled products was demonstrated by inoculated pack studies with *Staphylococcus aureus* and *Bacillus cereus*. The radiation sensitivity of *S. aureus* and *B. cereus* in the commercial meat products was initially investigated. The D_{10} values of *S. aureus* in mutton shami kebabs and chicken chilli were 0.33 ± 0.03 and 0.37 ± 0.03 kGy, respectively. The D_{10} values of *B. cereus* in mutton shami kebabs and chicken chilli were 0.47 ± 0.07 and 0.47 ± 0.08 kGy, respectively. *S. aureus* (inoculated 10^6 CFU/g) was eliminated at a dose of 2.5 kGy in both products, whereas *B. cereus* was eliminated at 3 kGy. The growth of both the test organisms inoculated into these products during storage at chilled temperatures (0–3°C and 10°C) was studied. No

growth of the test organisms was observed at 0–3°C. However, at higher temperatures such as 10°C, the organisms could multiply. These results indicated that temperature abuse during the storage of irradiated products should not be allowed. Radiation processing in combination with low temperature (0–3°C) storage thus resulted in microbiologically safe ready-to-eat products with extended shelf life.

1. INTRODUCTION

With rapid urbanization and change in socioeconomic status, there has been an increase in demand for convenience/ready-to-cook/ready-to-eat foods. Ready cooked meals, which are stored frozen at –18°C or below, are available in developed countries. These foods are not always safe, due to contamination by pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Aeromonas hydrophila* and *Escherichia coli* O157:H7 [1]. In India, meals are consumed within a short period after cooking. However, a number of ready-to-eat meal components such as lentils (dals) and vegetables with retort processing have started appearing in Indian markets. A retort process, such as freezing, is high in energy consumption and therefore costly. The ready-to-eat meal market is becoming increasingly popular among consumers looking for high quality and safe products. A developing country such as India can ill afford high energy consumption storage technology as it is not economically viable. Consumers prefer chilled foods over frozen as these are perceived to be fresh and more convenient. Freezing facilities are expensive. Also, freezing affects the texture of food and is not always safe as it does not eliminate pathogens. Ready-to-eat chilled meals are therefore of particular interest to India and other developing countries. However, cook–chill ready meals have a short shelf life of 7 d maximum at 4°C, thereby limiting the geographical area in which they can be marketed. Furthermore, there are significant concerns about their microbiological safety [2]. Cooking eliminates vegetative pathogens but sometimes undercooking may allow some of them to survive. In addition, there is always a risk of recontamination after cooking. *L. monocytogenes* is of particular concern being psychrotrophic and can grow at chilled temperatures. Technologies that allow a several fold extension of shelf life are therefore required. Radiation processing (1–2.5 kGy) of cook–chill meals at the end of the manufacturing process will result in safer food. The advantages of the process are that it makes these foods not only safe by eliminating non-sporulating pathogens but also extends the shelf life by decreasing the number of spoilage bacteria. Radiation processing has the potential to ensure microbiological safety of ready-to-eat foods without jeopardizing their taste or nutritive value.

There are ample data to show that irradiation at 2–5 kGy could significantly reduce the level of contaminants, thus improving the safety of individual food commodities. However, data are scanty on the effect of radiation processing of complex ready-to-eat meals, especially with regard to ethnic preparations. Irradiation could be useful for the safety of ready meals provided appropriate components are selected as ingredients, as some of the ingredients may show undesirable changes upon irradiation. Therefore, the present studies were undertaken to examine the effectiveness of irradiation for extending shelf life and improving the microbiological safety of some of the ethnic preparations that include items of a breakfast menu, meal components and complete meals.

In urban Indian markets several ready-to-eat meat products such chicken chilli, chicken tikka, mutton shami kebabs and mutton shish kebabs are marketed only in the frozen state. Storage of these products in a chilled state would be advantageous. The effect of radiation processing on the microbiological, chemical and sensory attributes of two popular ethnic Indian meat products (chicken chilli and mutton shami kebabs) commercially available in India was evaluated to ascertain their ultimate keeping quality.

The radiation doses required to render inactivate 90% of the CFU of the common foodborne pathogens associated with meat and meat products are in the range 1–4 kGy [3]. There are several reports on the radiation processing of meat products such as bacon, ham [4], sausages [5] and beefburgers [6]. The presence of bacteria of public health significance in ready-to-eat meat products is a known hazard to consumers as these products are sometimes consumed without thorough cooking. The safety of radiation processed products was also demonstrated using inoculated pack studies with *S. aureus* and *B. cereus*.

2. MATERIALS AND METHODS

2.1. Preparation of ready-to-eat meals

Ready-to-eat meals and meal components were prepared with the ingredients listed in Table 1. Commercially available chicken chilli and mutton shami kebabs were purchased from a local meat processor and brought to the laboratory in ice. For each product, a 100 g sample was packed in a sterile, low density polyethylene bag (700 gauge; WVTR 0.4 $g \cdot m^{-2} \cdot d^{-1}$; OTR 1800 $mL \cdot m^{-2} \cdot d^{-1}$). For sensory evaluation, a 200 g sample was packed separately. Three sets of experiments were carried out for each product.

TABLE 1. DESCRIPTION OF READY-TO-EAT MEALS

Item	Ingredients	Remarks
Breakfast:		
Poha	Dried pounded rice, onion, spices, vegetable oil	Warm and serve, a few drops of fresh lime juice enhance its taste
Upma	Semolina, onion, spices, vegetable oil	Warm and serve with coconut chutney
Meal components:		
Mixed vegetables	Peas, beans, carrots, onion, ginger–garlic paste, spices, vegetable oil	To be consumed with rice/roti (Indian bread) after heating
Prawn masala	Deveined prawns, ginger–garlic paste, spices, vegetable oil	Warm and serve with rice
Rice	Rice	Serve with mixed vegetables/ chicken or prawn curry
Complete meals:		
Vegetable pulao	Rice, peas, French beans, carrots, onion, ginger–garlic paste, spices, vegetable oil	Serve hot with curd
Chicken biryani	Rice, chicken drumsticks, ginger–garlic paste, spices, vegetable oil	Serve hot with curd
Prawn pulao	Rice, deveined prawns, ginger–garlic paste, spices, vegetable oil	Serve hot and garnish with coconut flakes
Khichadi	Rice, lentils, spices, vegetable oil	A good meal for patients and those with stomach upset
Commercial samples:		
Chicken chilli	Chicken cubes, ginger, garlic, capsicum, spices, vegetable oil	Serve hot as a side dish
Mutton shami kebabs	Mutton mince, semolina, onion, chillies, ginger–garlic paste and spices	Serve hot after frying as a side dish

2.2. Irradiation

Irradiation of prepackaged products was carried out at melting ice temperatures (1–3°C) in a food package irradiator with a ^{60}Co source at a dose rate of 3 kGy/h. The samples received minimal doses of 1, 2 or 3 kGy with an overdose ratio of 1.3. Dosimetry was performed by a cerric–cerrous dosimeter calibrated against Fricke's dosimeter. Dosimetry comparison was carried out with national standards established by the Radiological Physics and Advisory Division of the Bhabha Atomic Research Centre. A non-irradiated sample served as control. Until irradiation was complete, the control samples were kept in ice. All samples were then stored at 0–3°C.

2.3. Microbiological analysis

The sample (25 g) in duplicate from the irradiated and the corresponding control were aseptically homogenized for 1 min with sterile saline in a Stomacher (Seward, United Kingdom). Appropriate serial dilutions of the homogenate were carried out. Media used for the microbiological analyses were purchased from HiMedia, India. The TBC was determined using plate count agar incubated at 30°C for 48 h. Selective and differential media used were Baird Parker's agar (37°C for 24 h) for enumeration of *Staphylococcus* spp., violet red bile agar (44°C for 24 h) for faecal coliforms, sulphite polymixin sulphadiazine agar (37°C for 24 h in an anaerobic jar) for enumeration of sulphite reducing *Clostridium* spp., and potato dextrose agar (30°C for 5 d) for yeast and moulds. The total aerobic spore count was also determined. For this, 5 mL of the 10% homogenate was heated at 80°C for 10 min, cooled and then serial dilutions plated on plate count agar (30°C for 48 h).

2.4. Measurement of lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) produced from lipid peroxidation were determined using the method of Alasnier et al. [7]. A 4 g portion of each sample was blended with 16 mL of 5% trichloroacetic acid and BHT (10 μg BHT/g of lipids). It was then filtered through a Whatman filter (No. 4). An equal amount of filtrate and 0.02M thiobarbituric acid was heated in a boiling water bath for 30 min, cooled and the absorbance measured at 532 nm. The amounts of TBARS were expressed as milligrams of malonaldehyde per kilogram of meat.

2.5. Sensory evaluation

The sensory attributes evaluated were appearance, flavour, texture and overall acceptability of the products using a 10 point numerical scale, where 10 corresponded to 'components characteristic of the highest quality', 9 to 'loss in part of fresh components but not distinguished by new characteristics', 7–8 to 'first significant change; degree of component change slight but consistently apparent', 5–6 to 'moderate degree of change; increased intensity (quantitative change) and/or occurrence of additional components (qualitative change), but normal characteristics still dominant', 3–4 to 'strong degree of change; abnormal components dominant in contrast to normal components; loss in palatability definite', 1–2 corresponded to 'intense degree of change' and 0 to 'too poor to evaluate'. Scores from 9–6 were considered acceptable. The panel consisted of 15–20 experienced members of staff who were familiar with the characteristics of the meals under test. Sensory analyses were carried out after steaming the samples for 1–2 min.

2.6. Inoculated pack studies

2.6.1. Test organisms

S. aureus ATCC 6538P was maintained on nutrient agar slants at 4°C. Cells were grown at 37°C for 16 h in brain heart infusion broth (HiMedia). The samples were centrifuged in a refrigerated centrifuge (4°C) (Sorvall RC 2) at 12 100g for 10 min and washed twice with sterile normal saline. The pellet was suspended in sterile saline to give a cell count of approximately 10^7 CFU/mL.

B. cereus MTCC 470 was grown in soybean casein broth (HiMedia) at 37°C for 16 h. The samples were centrifuged in a refrigerated centrifuge (4°C) (Sorvall RC 2) at 12 100g for 10 min and washed twice with sterile normal saline. The cell pellet was suspended in sterile saline to give a cell count of approximately 10^6 CFU/mL.

2.6.2. Inoculation of meat products

Mutton shami kebabs and chicken chilli were irradiated (5 kGy) to eliminate the background flora. Aseptically, a suspension (0.5 mL) of test culture was uniformly spread on the meat products (25 g). The samples were kept in a sterile workstation for 30 min to allow it to be absorbed. These were then packed in sterile stomacher bags.

2.6.3. Determination of D_{10} *values of the test organisms*

The inoculated packs were irradiated at various doses at melting ice temperature. Two packs were irradiated at each dose. The maximum dose employed for both the test organisms was 3.0 kGy. Non-irradiated samples were also stored at the same temperature until irradiation was complete.

The number of survivors at each dose was determined by plating onto appropriate agars. Media employed for determining counts of *S. aureus* and *B. cereus* were Baird-Parker agar and soybean casein digest agar (HiMedia), respectively. The logarithm of the bacterial count was plotted against the radiation dose received to determine the death rate of each organism. The D_{10} values (the dose required to inactivate 90% of a population) for each of the organisms were calculated as the reciprocal of the slope of the logarithmic portion of the inactivation curve.

Survival of the test organisms in meat products during storage at chilled temperatures was also determined. Meat packs inoculated with test organisms were prepared as described above. The packs were subjected to gamma irradiation at 0–3°C at a dose rate of 3.0 kGy/h. The samples received a minimum dose of 3.0 kGy. For experiments on storage studies, samples were stored at 0–3°C and 10–12°C. Samples from both irradiated and non-irradiated lots were analysed immediately after irradiation and subsequently at regular intervals during storage at chilled temperatures.

2.7. Statistical analysis

All data are expressed as mean ± SD. Differences between variables were tested for significance by one way ANOVA with Tukey's post-test using GraphPad InStat version 3.05 for Windows 95 (GraphPad software, San Diego, United States of America). Differences at $p < 0.05$ were considered to be significant and $n = 3$.

3. RESULTS AND DISCUSSION

3.1. Microbiological quality

The TBCs of poha, upma, meals and meal components are depicted in Tables 2 and 3. The initial total aerobic count in non-irradiated samples varied with the product and increased by 2–3 log cycles in many products in less than 14 d storage and the samples spoiled. In irradiated samples, there was a dose dependent reduction and at 2 kGy TBC was significantly lower or non-detectable in some

TABLE 2. MICROBIOLOGICAL QUALITY OF BREAKFAST AND MEAL ITEMS STORED AT 0–3°C[a]

Meal	Storage period (d)	Control			1 kGy			2 kGy		
		0	15	30	0	15	30	0	15	30
		lg CFU/g								
Poha[a]	TBC[b]	3.4	5.5	6.7	2.7	4.1	4.4	2.3	2.1	4.0
	SC[c]	3.2	3.7	3.6	2.6	2.2	2.7	2.3	2.1	2.2
Upma[a]	TBC	1.9	3.5	5.6	n.v.c.[d]	n.v.c	n.v.c.	n.v.c.	n.v.c.	n.v.c.
	SC	1.3	1.3	1.7	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
Mixed vegetable[a]	TBC	1.0	n.v.c.	3.6	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c. n.v.c.
	SC	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
Prawn masala[a]	TBC	2.2	2.3	2.3	1.5	1.5	1.7	n.v.c.	n.v.c.	n.v.c.
	SC	1.9	2.2	2.2	1.0	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
Rice[a]	TBC	1.0	2.6	5.6	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
	SC	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.

[a] *Staphylococcus* spp., fungus and faecal coliforms not detected.
[b] TBC: total bacteria count.
[c] SC: spore count.
[d] n.v.c.: no organisms detected by the method employed.

samples, even after 28 d storage. Fungal growth was detected in some of the non-irradiated samples during chilled storage. The enhanced safety of radiation processed products was evident from the complete elimination of potentially pathogenic *Staphylococcus* spp. Microbial load, in particular aerobic spore counts in poha, was fairly high and not completely eliminated with a dose of 2 kGy.

Non-irradiated vegetable pulao had an initial TBC and aerobic spore count of about 2–3 lg CFU/g. The effect of irradiation treatment on the microbiological quality was a reduction in total viable count, as well as levels of spores. Potentially pathogenic *Staphylococcus* spp. was absent in both irradiated as well as non-irradiated samples. During storage at 0–3°C for 30 d, total bacteria, *Staphylococcus* and aerobic spore counts gradually increased to 7.8, 3.9 and 4.37 lg CFU/g, respectively. Irradiated samples were microbiologically superior to their non-irradiated counterparts throughout the storage period. A TBC of 1.3 lg CFU/g was observed in a 1 kGy irradiated sample after storage for 30 d. No viable organisms were detected in samples treated with 2 kGy dose.

TABLE 3. MICROBIOLOGICAL QUALITY OF COMPLETE MEALS STORED AT 0–3°C[a]

Meal	Dose	Control			1 kGy			2 kGy		
	Storage period (d)	0	15	30	0	15	30	0	15	30
		lg CFU/g								
Vegetable pulao[a]	TBC[b]	2.6	6.5	6.4	n.v.c.[c]	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
	SC[d]	2.0	1.3	2.8	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
Chicken biryani[a]	TBC	2.9	3.0	4.3	2.4	2.3	1.6	2.3	2.2	2.0
	SC	1.5	2.7	2.4	2.0	1.3	1.7	2.1	1.8	n.v.c.
Prawn pulao[a]	TBC	2.7	3.7	5.6	1.0	1.5	n.v.c.	n.v.c.	1.3	n.v.c.
	SC	1.7	n.v.c.	1.7	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
Khichadi[a]	TBC	1.6	1.8	n.d.[e]	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
	SC	n.v.c.	n.v.c.	n.d.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.

[a] *Staphylococcus* spp., fungus and faecal coliforms not detected.
[b] TBC: total bacteria count.
[c] n.v.c.: no organisms detected by the method employed.
[d] SC: spore count.
[e] n.d.: fungus visible and consequently sample not analysed.

Non-irradiated chicken biryani had initial total bacteria and aerobic spore counts of about 2.9 and 1.5 lg CFU/g, respectively. The immediate effect of irradiation treatment on the microbiological quality was a reduction in TBC as well as levels of spores. Total bacteria, *Staphylococcus* and aerobic spore counts gradually increased to 4.26, 3.48 and 2.88 lg CFU/g, respectively, during storage at 0–3°C for 30 d (Tables 2 and 3). In irradiated samples, the radiation damaged organisms failed to multiply rapidly, hence the counts observed were almost static. This could be attributed to a combination of radiation damage, chilled storage and the presence of spices, which make the environment hostile for multiplication of organisms.

The TBCs of chicken chilli and mutton shami kebab are shown in Figs 1 and 2, respectively. Results are the mean values of three independent experiments.

Irradiation significantly ($p < 0.05$) improved the microbiological quality of the products by reducing the TBC. Further, the decrease in TBC was dose dependent in both of the products. The numbers increased with storage time and there was a significant ($p < 0.05$) difference between the irradiation doses. In less than 14 d, non-irradiated chicken chilli had counts greater than

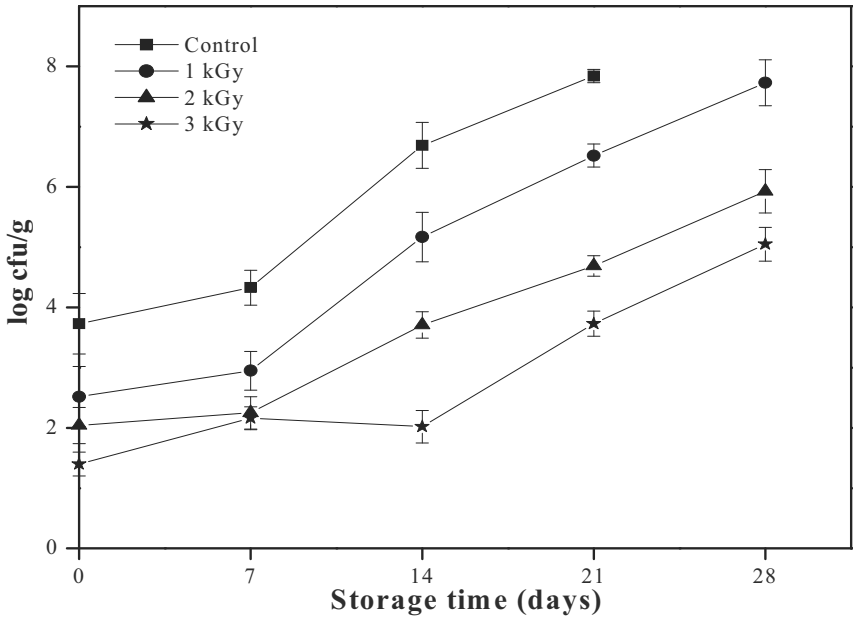

FIG. 1. Effect of irradiation (up to 3 kGy) on the TBCs of chicken chilli during storage at 0–3°C. Vertical lines represent error bars.

6 lg CFU/g, while in irradiated samples (3 kGy) it did not reach this value even after 28 d. In the case of mutton shami kebabs, control samples spoiled in less than a week while in irradiated samples after 28 d storage at 0–3°C, the counts were less than 4 lg CFU/g. The effect of low dose irradiation in reducing the bacterial load of meat and meat products has been reported earlier from the authors' laboratory [8, 9]. The decrease in total bacterial population as a result of irradiation was in agreement with other studies [10]. Under aerobic conditions, the dominant spoilage organisms are the strictly aerobic *Pseudomonads* [11], which are very sensitive to irradiation [12]. The bacteria that grow most rapidly under the storage conditions of the products usually dominate the spoilage flora of meat products. The sensitivity of these organisms to radiation explains the decline caused in the total aerobic counts of the irradiated products. The D_{10} value of 0.13 kGy at 5°C for *P. fluorescens* in beef has been reported [13].

Radiation processing had a significant effect on the reduction/elimination of pathogenic bacteria (Table 4).

In both products, irradiation at 2 and 3 kGy resulted in complete elimination of aerobic spore counts and *Staphylococcus* spp.

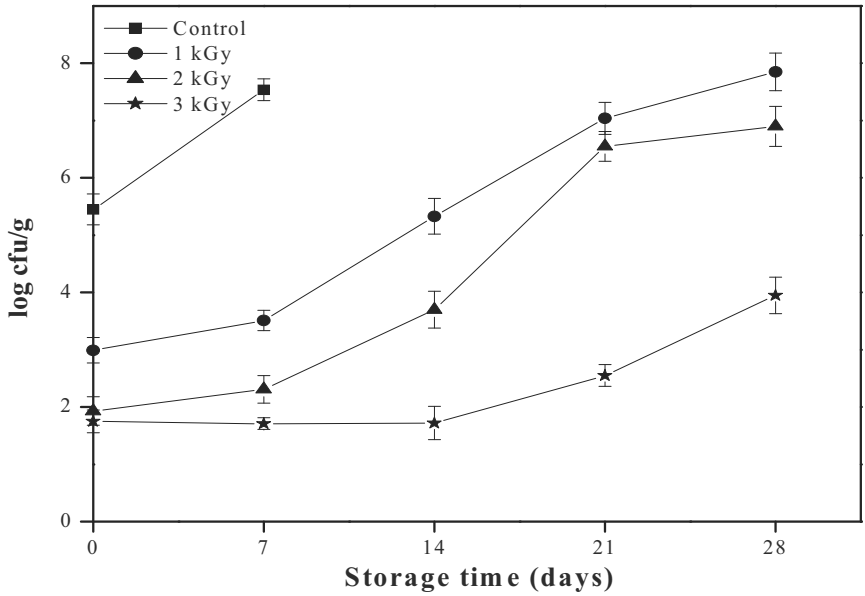

FIG. 2. Effect of irradiation (up to 3 kGy) on the TBCs of mutton shami kebab during storage at 0–3°C. Vertical lines represent error bars.

TABLE 4. TOTAL *Staphylococcus* spp. AND AEROBIC SPORE COUNTS OF MEAT PRODUCTS STORED AT 0–3°C

Sample	Storage time (d)				
	0	7	14	21	28
Total *Staphylococcus* spp. counts (lg CFU/g):					
Chicken chilli (control)	2.32	3.02	3.59	5.12	n.d.[a]
Chicken chilli (1 kGy)	n.v.c.[b]	n.v.c.	n.v.c.	n.v.c.	n.v.c.
Mutton shami kebabs (control)	4.17	4.58	n.d.	n.d.	n.d.
Mutton shami kebabs (1 kGy)	1.43	1.43	1.51	1.52	1.6
Total aerobic spore counts (lg CFU/g):					
Chicken chilli (control)	1.12	1.21	1.14	1.42	n.d.
Chicken chilli (1 kGy)	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
Mutton shami kebabs (control)	2	2	n.d.	n.d.	n.d.
Mutton shami kebabs (1 kGy)	1.6	2	2	2.2	2

[a] n.d.: analysis not undertaken due to sample spoiling.
[b] n.v.c.: no organisms detected by the method employed.

In chicken chilli, non-irradiated control samples had initial *Staphylococcus* spp. counts of 2.32 lg CFU/g, which increased to 5.12 lg CFU/g after 21 d. In all irradiated chicken chilli samples, *Staphylococcus* spp. was not detected throughout the storage period. About 4 lg CFU/g of *Staphylococcus* spp. was present in non-irradiated mutton shami kebabs and irradiation at 1 kGy reduced the *Staphylococcus* spp. counts by 2 log cycles. In samples irradiated at 2 and 3 kGy, this organism was not detected throughout the storage period. It has already been reported that 90% of *S. aureus* in mechanically deboned chicken meat is killed by a dose of 0.36 kGy [14]. The D_{10} values in the range 0.40–0.46 kGy for *S. aureus* in different meat systems irradiated at 5°C have also been reported [15].

Faecal coliforms were detected in only one batch of non-irradiated control chicken chilli samples. Faecal coliforms were not detected in any of the irradiated samples. Similar findings have been reported earlier by Naik and others [8]. They found that irradiated meat (2.5 kGy) was completely free of *Enterobacteriaceae* for the entire storage period (0–3°C) of five weeks. Irradiation of fresh pork had the maximum effect on *Enterobacteriaceae* [16]. A minimum dose of 1.5 kGy would destroy at least 6 lg of *E. coli* O157:H7, which has a D_{10} value of about 0.24 kGy [17].

Of concern in any processed meat product is the survival and growth of spore formers. Sulphite reducing *Clostridia* were not detected in any of the samples throughout the storage period. The aerobic spore count in non-irradiated chicken chilli was 1.12 lg CFU/g and these organisms were not detected in any of the irradiated samples (Table 4). In the control mutton kebabs a spore count of 2.7 lg CFU/g was measured and even after irradiation some low counts were found in irradiated samples. Lambert et al. [16] have also reported that aerobic spore formers were present at levels of 10–2.5×10^3/g in some fresh pork samples irradiated at 0.5 kGy and stored at 15°C. Yeast/moulds were not detected in any of the samples throughout the storage period. Among the most sensitive microorganisms to radiation are gram negative rods, followed by gram positive cocci and rods, yeast, moulds, fungal spores, aerobic and anaerobic spore formers [11]. Low dose irradiation kills microorganisms of public health significance and extends the shelf life of meat products. This has already been established in earlier studies [3, 4] and the present study further confirms these findings for ethnic Indian meat products.

3.2. Lipid peroxidation

Lipid peroxidation was measured in terms of TBARS. Non-irradiated control samples showed lower TBARS values than irradiated samples. The

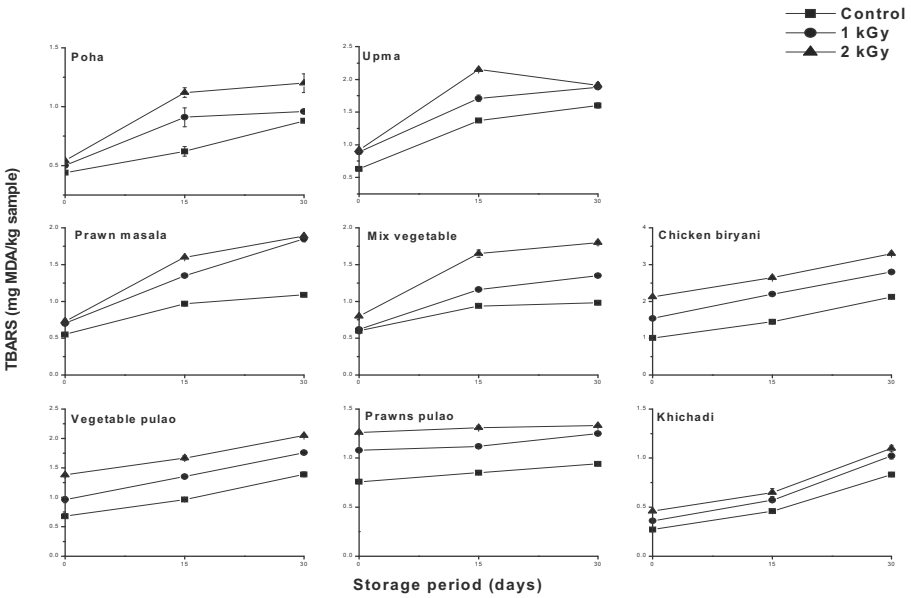

FIG. 3. Lipid peroxidation measured as TBARS value of radiation processed meals. Vertical lines represent error bars.

increase in the TBARS value depended on the dose and on the period of storage (Fig. 3).

TBARS values of chicken chilli and the kebabs are shown in Figs 4 and 5. Results are the mean values of three independent experiments.

Non-irradiated control samples showed lower TBARS values ($p < 0.05$) than irradiated samples. In the case of chicken chilli, the increase in the TBARS value of irradiated samples was not very significant ($p > 0.05$). This may probably be due to the spices used in its preparation that are known to have antioxidant activity. The increase in the TBARS value was dose dependent. Several other workers have also reported the acceleration of lipid oxidation caused by radiation processing [18–20]. The presence of oxygen is the most critical factor influencing lipid oxidation during the storage of irradiated meat [21]. Lipid oxidation and discoloration of meat is enhanced in the presence of oxygen while increasing the antimicrobial action of radiation. Hence, irradiating meat in a frozen state or in modified atmosphere packing/ vacuum packaging or by addition of antioxidants can minimize or avoid the development of rancidity.

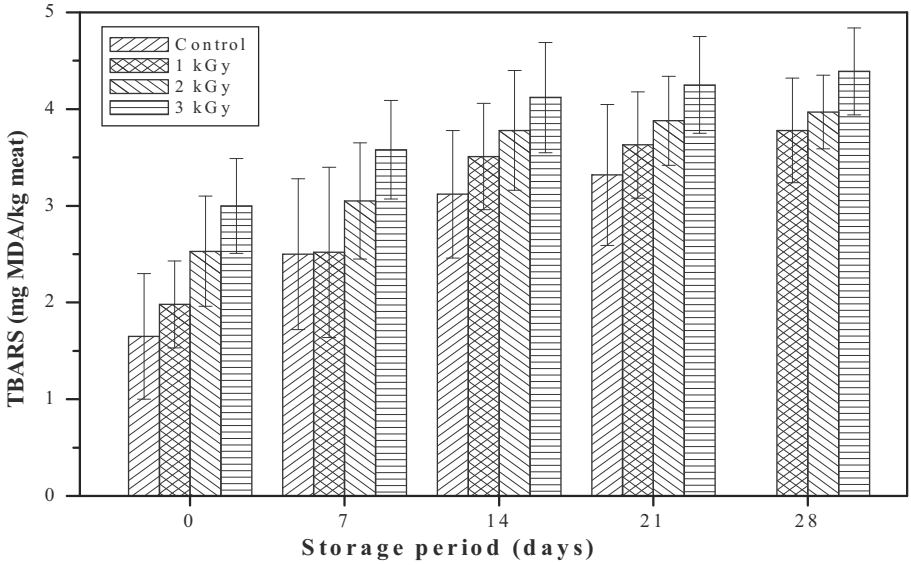

FIG. 4. TBARS values of chicken chilli during storage (0–3°C). Vertical lines represent error bars.

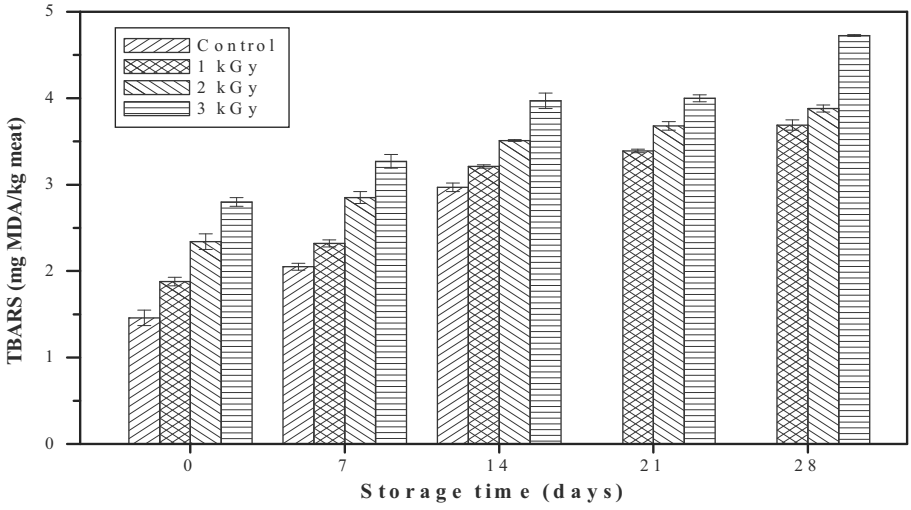

FIG. 5. TBARS values of mutton shammi kebab during storage (0–3°C). Vertical lines represent error bars.

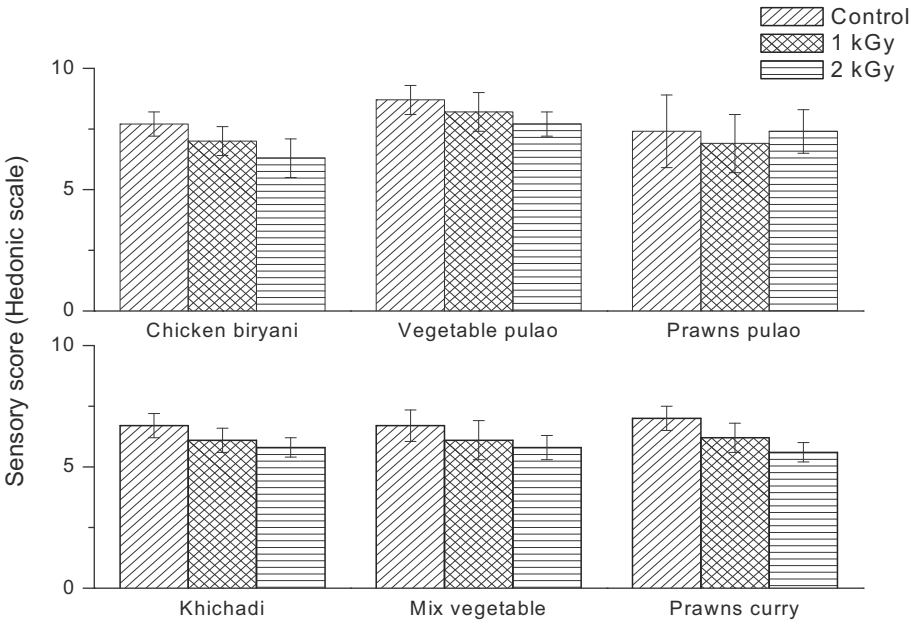

FIG. 6. Sensory evaluation of radiation processed meals. Vertical lines represent error bars.

3.3. Sensory evaluation

The appearance, flavour and texture of irradiated samples were not different from their non-irradiated controls, and all the samples were acceptable (data not shown). The overall sensory scores of irradiated and non-irradiated samples were not significantly different (Fig. 6).

The results of the initial sensory evaluations carried out for the meat products are illustrated in Fig 7. Results are the mean values of three independent experiments.

For safety reasons, the samples were not submitted to the panellists if their microbiological examination revealed a count of 10^6 CFU/g or more. In the case of chicken chilli it was found that immediately after irradiation the overall sensory scores of irradiated and non-irradiated samples were not significantly ($p < 0.05$) different. Appearance, flavour and texture of irradiated samples were not different from their non-irradiated controls and all the samples were acceptable. The same observation was made in the case of mutton shami kebabs (Fig. 7). Though oxidative rancidity measured in terms of the TBARS value increased on irradiation, there was no significant effect on

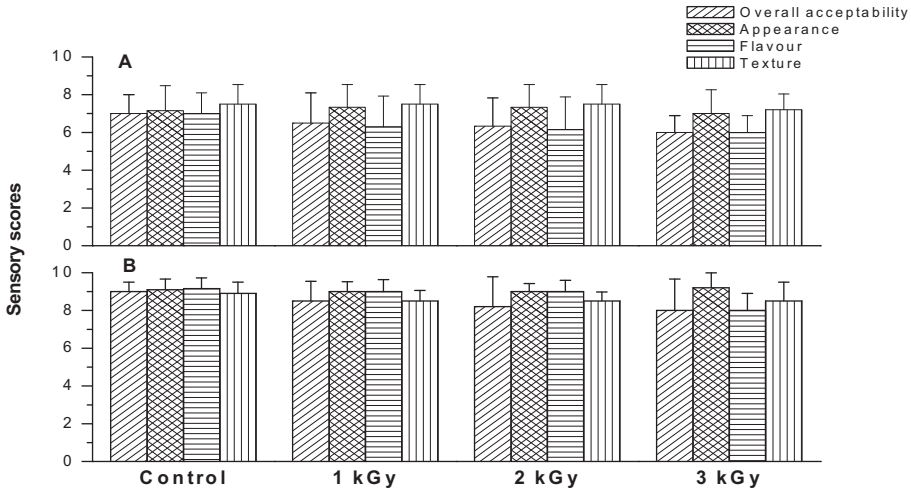

FIG. 7. Sensory evaluation of (A): mutton shami kebab and (B): chicken chilli. Vertical lines represent error bars.

the sensory quality of the irradiated meat. For irradiation of the three meat products, the doses employed (1, 2 and 3 kGy) were either below or close to the threshold level and hence no irradiation odour was detected. Irradiation with a dose above the threshold level had been reported as producing irradiation odour in various meats [22]. As the control samples spoiled in a week they were not used for further sensory analysis. Irradiated (3 kGy) samples were found to be acceptable even at the end of the storage period (Fig. 8).

Results are the mean values of three independent experiments.

The authors' observation is in agreement with the earlier findings of Kiss and Farkas [23] who reported that irradiation (2–5 kGy) extended the keeping quality of chicken carcasses in cold storage by a factor of two to three (10–15 d longer than the control), without noticeable deterioration in organoleptic quality. Irradiated meat will be successful in the market place only if consumers are satisfied with its sensory quality.

3.4. Inoculated pack studies

3.4.1. Radiation sensitivity of test microorganisms

The effect of radiation treatment on the survival of *S. aureus* and *B. cereus* in mutton shami kebabs and chicken chilli is shown in Fig. 9.

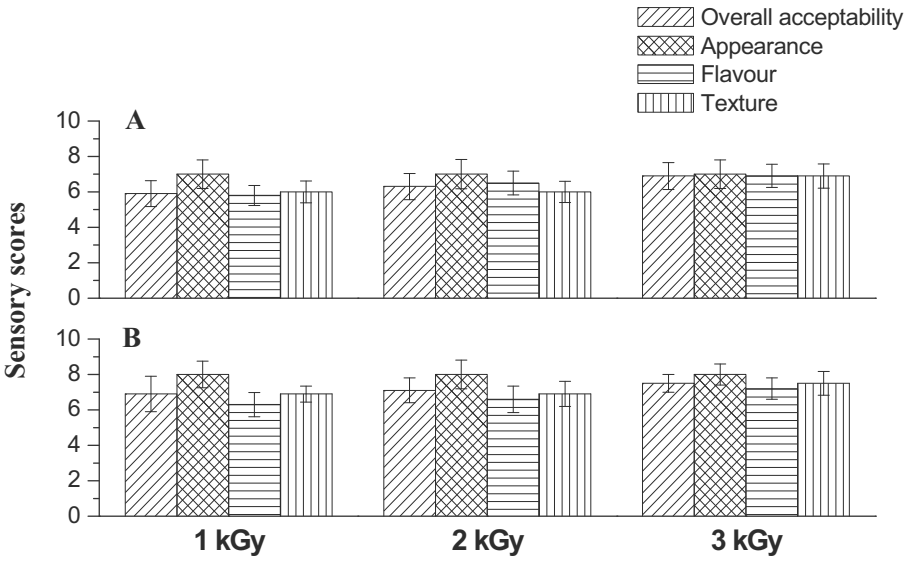

FIG. 8. Sensory evaluation of (A): mutton shami kebab and (B): chicken chilli after storage for 4 weeks at 0–3°C. Vertical lines represent error bars.

The D_{10} values of *S. aureus* were 0.33 ± 0.03 and 0.37 ± 0.03 kGy in mutton shami kebabs and chicken chilli, respectively. Similarly, in the case of *B. cereus,* the D_{10} values were 0.47 ± 0.07 and 0.47 ± 0.08 kGy in mutton shami kebabs and chicken chilli, respectively.

The radiation sensitivity of bacteria is affected by a number of factors such as the medium of suspension, water activity, composition, irradiation temperature and the presence of oxygen [24]. In their previous studies, the authors observed D_{10} values for *S. aureus* and *B. cereus* suspended in sterile normal saline to be 0.14 kGy and 0.16 kGy, respectively [25]. The D_{10} values for bacteria are normally higher in the presence of food than in saline. In roast beef meal components, D_{10} values in the range 0.25–0.37 kGy and 0.13–0.29 kGy for *S. aureus* and *B. cereus,* respectively, have been reported [26]. Thayer and others [15] reported D_{10} values in the range 0.40–0.46 kGy for *S. aureus* in different meat systems irradiated at 5°C. In mutton kebabs, D_{10} values for *S. aureus* and *B. cereus* are reported as 0.36 kGy and 0.29 kGy, respectively [25]. Similarly, in Kwamegi (traditional Korean semi-dried fish), D_{10} values for *S. aureus* and *B. cereus* were reported as 0.59 kGy and 0.64 kGy, respectively [27]. The authors' results are comparable with these studies. Variations in D_{10} values can be attributed to the difference in irradiation temperature and in the suspension medium.

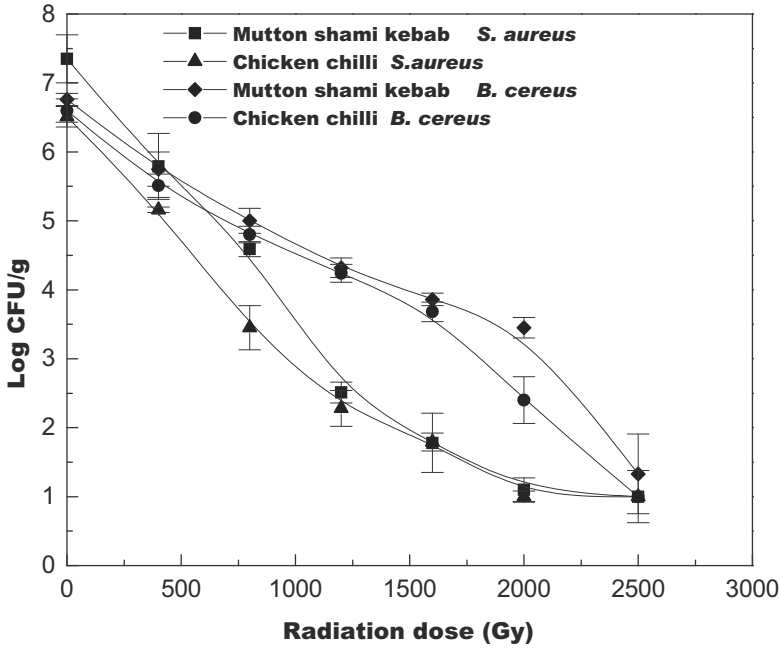

FIG. 9. Effect of gamma irradiation treatment on survival of S. aureus and B. cereus in mutton shami kebabs and chicken chilli. Vertical lines represent error bars.

The higher resistance of bacteria in foods can be attributed to various food components. As in complex food systems, some of the constituents such as proteins are believed to scavenge radiolytic free radicals, thereby reducing the net effect of radiation damage and making the organism more resistant [28].

The viability patterns of *S. aureus* and *B. cereus* during chilled storage at 0–3°C and 10–12°C in mutton shami kebabs and chicken chilli are shown in Tables 5 and 6, respectively.

It was observed that in the case of non-irradiated samples stored at 0–3°C, viable counts of *S. aureus* and *B. cereus* remained stationary at about 10^6 CFU/g and 10^4 CFU/g, respectively, during the storage period of 4 weeks (Table 5). In irradiated samples, no viable cells of either test organism were detected during the study period. However, when storage temperature was increased to 10–12°C in the case of non-irradiated samples, viable counts of *S. aureus* and *B. cereus* increased by 2–3 log cycles over the storage period of 4 weeks (Table 6). In irradiated samples, no viable cells of the test organisms were detected during the study period. The minimum growth temperature for *S. aureus* and *B. cereus* is reported as 6°C [29] and 5°C [30], respectively. The results of the present study confirm these findings and propose the importance of temperature

TABLE 5. VIABILITY OF *S. aureus* AND *B. cereus* IN MEAT PRODUCTS STORED AT CHILLED TEMPERATURE (0–3°C)

Product	Initial	1 week	2 weeks	3 weeks	4 weeks
			S. aureus counts (lg CFU/g)		
Mutton shami kebab (control)	6.1 ± 0.24	6.3 ± 0.29	6.2 ± 0.37	6.9 ± 0.29	6.9 ± 0.39
Mutton shami kebab (3 kGy)	n.v.c.[a]	n.v.c.	n.v.c.	n.v.c.	n.v.c.
Chicken chilli (control)	5.6 ± 0.14	5.7 ± 0.21	5.6 ± 0.10	5.9 ± 0.25	5.8 ± 0.52
Chicken chilli (3 kGy)	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
			B. cereus counts (lg CFU/g)		
Mutton shami kebab (control)	4.2 ± 0.64	4.3 ± 0.44	3.9 ± 0.36	3.8 ± 0.63	3.8 ± 0.46
Mutton shami kebab (3 kGy)	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
Chicken chilli (control)	4.3 ± 0.25	4.0 ± 0.35	4.1 ± 0.41	3.9 ± 0.76	3.9 ± 0.66
Chicken chilli (3 kGy)	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.

[a] n.v.c.: no viable organisms detected by the method employed.

TABLE 6. VIABILITY OF *S. aureus* AND *B. cereus* IN MEAT PRODUCTS STORED AT CHILLED TEMPERATURE (10–12°C)

Product/treatment	Initial	1 week	2 weeks	3 weeks	4 weeks
			S. aureus counts (lg CFU/g)		
Mutton shami kebab (control)	5.68 ± 0.78	6.01 ± 0.02	7.64 ± 0.65	n.d.[a]	n.d.
Mutton shami kebab (3 kGy)	n.v.c.[b]	n.v.c.	n.v.c.	n.v.c.	n.v.c.
Chicken chilli (control)	5.43 ± 0.95	6.02 ± 0.11	7.51 ± 0.38	n.d.	n.d.
Chicken chilli (3 kGy)	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
			B. cereus counts (lg CFU/g)		
Mutton shami kebab (control)	5.02 ± 0.47	5.70 ± 0.43	7.89 ± 0.75	n.d.	n.d.
Mutton shami kebab (3 kGy)	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
Chicken chilli (control)	5.05 ± 0.17	5.74 ± 0.49	7.23 ± 0.47	n.d.	n.d.
Chicken chilli (3 kGy)	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.

[a] n.d.: analysis not undertaken due to sample spoiling.
[b] n.v.c.: no viable organisms detected by the method employed.

control during storage and marketing of chilled ready-to-eat meat products, in ensuring the efficacy of irradiation treatment for enhancing the microbiological safety of ready-to-eat meat products.

4. CONCLUSION

Radiation processing of ethnic Indian ready-to-eat products resulted in a dose dependent decrease in the total viable counts and in the reduction/elimination of pathogenic organisms. Irradiation at 2–3 kGy extended the chilled storage life of the ready-to-eat products by 2 weeks compared with non-irradiated samples. The results of packed inoculum study demonstrated that irradiation in conjunction with chilled storage can enhance the microbiological safety of ready-to-eat food products. Thus, radiation processing could be used to the advantage of processors, retailers and consumers.

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IRRADIATION TO ENSURE THE SAFETY AND QUALITY OF SOME ETHNIC SOUPS, SNACKS AND YUNAN CHICKEN

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Abstract

Semi-concentrated black, oxtail, chicken vegetable and chicken sweetcorn soups, precooked spring roll, rissole and croquette snacks, and Yunan chicken were individually packed in a dry laminate pouch of PET 12 μm/LDPE adh.2 μm/Al foil 7 μm /LDPE adh/LLDPE (C_4) 50 μm under vacuum followed by freezing for 24 h at –18°C prior to irradiation with doses of 1, 3, 5 and 7 kGy at cryogenic conditions (–79°C). Both the non-irradiated and irradiated prepared meals were then stored in a refrigerator at 5 ± 2°C. The non-irradiated samples and those samples irradiated at 1 kGy were mostly damaged after a week in storage. Gamma irradiation at doses of 5–7 kGy for soups and snacks, and doses of 3–5 kGy for Yunan chicken can reduce the microbial load by about 2–3 log cycles, respectively, without affecting the physicochemical parameters and palatability over 2–3 months. However, the irradiated spring roll was unable to withstand more than 1 month storage. The D_{10} values for potential pathogens on Yunan chicken were 0.28 kGy for *Salmonella typhimurium*, 0.17 kGy for *Pseudomonas aeruginosa,* 0.12 kGy for *Escherichia coli* O157, 0.66 kGy for *Listeria monocytogenes* and 0.09 kGy for *Campylobacter jejuni*.

1. INTRODUCTION

A successful experiment on the application of irradiation technology at high doses in combination with storage under cryogenic conditions in the processing of some vacuum packed Indonesian ethnic dishes concluded that gamma irradiation at the dose of 45 kGy could maintain the safety and quality attributes for 1.5 years at 28–30°C [1, 2]. The purpose of radiation processing at

such a high dose is that such meals are meant for consumption by a hospital patient, people engaging in outdoor activities and military personnel. Such meals would offer convenience and shorter preparation time, but conventionally stored, with limited shelf life under ambient temperatures, in a chilled or frozen state for long term sale in supermarkets and intended for microwaving prior to consumption.

Reportedly, irradiation at pasteurization doses also has a potential role in improving the microbiological safety and shelf life of prepared meals [3–6]. The prepared meals currently marketed under frozen conditions could possibly be replaced through irradiation followed by storage under chilled conditions. This would ensure safety, maintain quality, based on objective and subjective parameters, and lengthen shelf life, thereby meeting market requirements and resulting in more practical and quick preparation, thereby saving energy and lowering operation cost.

The overall objective of this study was to evaluate the effectiveness of medium irradiation doses in ensuring the microbiological safety and to extend the shelf life within months of some ethnic prepared meals and functional foods such as semi-concentrated soups, snacks, and Yunan chicken, being vacuum packed in a laminate pouch and stored at 5 ± 2°C. The microbiological assessments, some physicochemical characteristics and their acceptability from the organoleptic point of view as well as sensory attributes of the treated products and determination of D_{10} values for some pathogenic bacteria in Yunan chicken were also carried out.

2. MATERIALS AND METHODS

2.1. Materials

Four selected frozen soups: black, oxtail, chicken vegetable and chicken sweetcorn, were purchased from a supermarket, while precooked snacks: spring rolls, rissole, croquette and Yunan chicken, were sourced locally. Commercially, around 400 cm^3 of each type of the soup was individually wrapped in a high density polyethylene (HDPE) pouch. Snacks were divided into groups, each group containing 6 pieces of each type of snack which were wrapped with the same type of plastic pouch. The waxy thin carton was selected as a secondary packing for each product, then it was kept in frozen conditions, at –18°C, until it sold out. After arrival in the laboratory, the boxes of soups and snacks were stored at –18°C prior to use for the research work. Similar types of soup, i.e. black, oxtail, chicken vegetable and chicken sweetcorn, were also prepared and developed at the BATAN laboratory in parallel to provide a

formulation for a standard operating procedure for irradiating soups. The quantity of raw materials used in making the selected soups in the laboratory to develop a standard operating procedure is listed in Annex I, and the quantity of raw materials used by the food industry in making the selected snacks is listed in Annex II.

2.2. Methods

2.2.1. Laboratory preparation of selected soups

The raw materials used in making the soups are listed in Annex I.

2.2.1.1. Rawon/black soup

Beef was boiled in 5 L of water for 1 h then removed and cut into 2 × 2 × 2 cm cubes. Shallot, garlic, candlenut, coriander, red chilli and *Pangium edule* were ground in a blender to form a mix of ingredients. Palm oil was heated and then mixed with the ingredients, followed by addition of roasted fish paste, turmeric, lemon leaf, ginger root and lemon grass until the the aroma manifested itself. The beef was added to the mixture and fried for 10 min. An amount of broth, 50% taken from the total volume, was then poured into the spicy cooked beef and added with some salt and sugar. The soup was ready after cooking for 30 min.

2.2.1.2. Oxtail soup

Oxtail was boiled in water for 1 h. The oxtail was then removed from the broth and cut into 2 cm thick pieces. The oxtail was then put into heated palm oil for 5 min, removed, and put onto a separate plate. All the ingredients, together with cut onion, onion leaf and celery, were fried in margarine. An amount of broth, 50% from the total volume, was then poured onto the fried ingredients and vegetables, then cooked together with the oxtail for 15 min. Ground white pepper and nutmeg were finally added into the mixture 30 min prior to cooking.

2.2.1.3. Chicken vegetable soup

Chicken was first boiled in 3 L of water, removed, and then cut into cubes. Margarine was first heated in a pan, followed by the ground ingredients, celery and onion leaf until an aroma arose. Chicken broth was boiled and added to the fried ingredients and boiled for 30 min. The broth, 50% from the total volume,

was separated from the ingredients and boiled again. Carrot (0.5 cm slices), green beans, broccoli, defrosted sugar peas, chopped chicken and ground white pepper were added to the broth and boiled for 20 min.

2.2.1.4. Chicken sweetcorn soup

Chicken was first boiled in 3 L of water, removed, and then cut into cubes. After removing the corn from the stalk, part of the corn was mixed with the chicken broth; some salt, sugar, ground white pepper, ground nutmeg were then added and kept boiling for 15 min. Corn starch was added to the cooked mixture, together with chicken meat, chicken sausage (0.5 cm thick slices), carrot and corn (not blended), and boiled again for 15 min. At the end, beaten eggs were poured into the final cooked mixture and boiled for another 15 min.

2.2.2. Local preparation of selected precooked snacks and Yunan chicken

2.2.2.1. Spring roll

Filling: Both the sliced shallot and garlic were fried in vegetable oil until the aroma arises. The rest of the filling materials as listed in Annex II were added, mixed thoroughly, decanted, and then split into 20 portions.

Sweet sauce and tauco sauce: All materials listed in Annex II were mixed and allowed to boil in order to cook and raise the consistency. The sauce was not irradiated but served separately.

Finally, the spring roll was prepared by spreading the cooked filling on top of a spring roll sheet, folding it into a rectangular shape, and the edges with sealed with egg.

2.2.2.2. Rissole

Rissole sheet: Rissole was prepared by pouring the materials into a bowl, whisking and mixing thoroughly. The mix was then fried in a pan and made into a very thin sheet.

Filling: The procedure for cooking the filling was similar to that for spring rolls, but the materials used in this preparation are different from those listed in Annex II. As final preparation, the filling was poured onto a piece of sheet, folded into an envelope shape, and the edges sealed with egg. Finally, the rissole was rolled on breadcrumbs until the surface was completely covered.

2.2.2.3. Croquette

Mashed potato, together with the other ingredients, were prepared as a croquette wrapper (the ingredients are listed in Annex II). The rest of the method of making croquettes was the same as that for making rissoles.

2.2.2.4. Yunan chicken

Yunan chicken was also selected and prepared by a local establishment, but the general recipe for making the chicken was given by another source [7]. It was prepared from 200–225 g body weight of a 3 month-old hen ('buras'). The chicken was slaughtered, washed, then marinated for 40–60 min in various spices such as ginger, garlic, pepper, Chinese ingredients which were prepared from dried plants and then ground, with sufficient water added. The whole marinated chicken was then wrapped with salt paper and bound tightly followed by addition of salt, then cooked in a stainless steel pan for 3 h at low heat, removed and finally wrapped with HDPE sheet.

2.2.3. Packaging material and sample condition

The packing technique was confirmed by the quality assurance guidance manual model for safe shelf-stable foods using high dose irradiation [8]. A type of laminated pouch of PET 12 μm/LDPE as adh. 2 μm/Al foil 7 μm/LDPE as adh./LLDPE 50 μm was selected similar to the previous work [1] as the primary packaging of 'warm fill' soups, snacks and Yunan chicken prior to being vacuum sealed. Styrofoam boxes (33.75 cm × 36.25 cm × 51.25 cm) were filled with dry ice at –79°C, in order to maintain the cryogenic condition of the prepared meals during the irradiation process [9].

2.2.4. Irradiation treatment and storage condition

All non-irradiated and irradiated prepared meals were kept at –20ºC for 48 h before removing to styrofoam boxes. Gamma irradiation was conducted at the IRPASENA irradiator at the Centre for the Application of Isotope and Radiation Technology, National Nuclear Energy Agency, Pasar Jumat, Jakarta. Cobalt-60 was used as the source of ionizing radiation (capacity of 20 kCi) at a dose rate of 3 kGy/h. Perspex was used as the calibration dosimeter to determine absorbed dose [10]. Soups and snacks were irradiated with absorbed doses of 1, 3, 5, 7 kGy and doses of 3 and 5 kGy for Yunan chicken, respectively.

Following irradiation, all non-irradiated and irradiated prepared meals were then stored at 5 ± 2ºC and tested for up to 3 months' storage for soups and

snacks and 9 weeks for Yunan chicken, in order to determine the effect of gamma irradiation on objective parameters such as microbial safety and radiation sensitivity of some pathogenic bacteria and physicochemical characteristics, and subjective quality parameters.

2.2.5. Methods of analysis and statistics

2.2.5.1. Microbiological safety of the prepared meals

The microbial load of the total bacteria plate count (TBC) (CFU/g) and total mould and yeast count (TMYC)(CFU/g) were assessed according to Indonesian National Standard methods [11] and Buckle [12], respectively. *Staphylococcus aureus* was identified using the Indonesian National Standard method [13]. The presence of *Escherichia coli (E. coli)* and coliforms was calculated as the most probable number (MPN) [14], *Clostridium perfringens* was identified according to the previous method [2] and *Salmonella* spp. was done according to Australian Standard [15]. These microbes are of particular importance in the microbiological assessment of safe stable foods stored in a refrigerator [16].

2.2.5.2. Determination of D_{10} values for some pathogenic bacteria in Yunan chicken

The determination of D_{10} values for some potential pathogenic bacteria such as *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *E. coli* O157 on commercial Yunan chicken was developed from the method of Rashid et al. [17]. Three local type strains of *S. typhimurium*, *P. aeruginosa* and *E. coli* O157 were used to determine the D_{10} value in the sterilized chicken meat. The micro-organisms were propagated in nutrient agar medium (containing Bacto beef extract 3 g, Bacto peptone 5 g and agar 15 g) and incubation was conducted at 37°C for 24–48 h. The cells were harvested and placed in sterilized distilled water, then inoculated into the samples using a sterilized syringe. The inoculated samples were then irradiated with doses of 0.1, 0.2, 0.3 and 0.4 kGy, respectively. Fricke dosimetry was conducted [10] and the dose rate was 1.14 kGy/h. The irradiated samples were plated by serial dilutions and then incubated at 37°C for 24–48 h.

Inoculation, enumeration and sample preparation to determine the D_{10} values for *Listeria monocytogenes* and *Campylobacter jejuni* were also developed [12, 14]. The freeze-dried culture of *L. monocytogenes* was a local isolate. It was initially grown on *Listeria* enrichment broth base (LEBB) (OXOID CM 862) for 48 h at 37ºC. The purity check of the isolate was done by

growing the isolate on blood agar with 5% defibrinated sheep blood. The pure culture was then grown in LEBB. For preparing the inoculum of *L. monocytogenes*, the growth curve of *L. monocytogenes* in LEBB was determined first by enumerating the bacterial concentration on blood agar plates and measuring the optical density (at 550 nm) of the culture broth. The desired concentration of *L. monocytogenes* (10^8 CFU/mL) was used as inoculum. Prior to inoculation, Yunan chicken was cut into pieces and made into 100 g packages. The chicken was first wrapped with aluminum foil, then packed in a sterile HDPE bag. The chicken packages were then autoclaved to eliminate all microorganisms in the chicken. Chicken inoculations with *L. monocytogenes* were done in biohazard class II cabinet at the Research Institute for Veterinary Science Laboratory in Bogor. Each package of 100 g was inoculated with 1 mL of culture broth containing approximately 10^8 CFU *L. monocytogenes*. Therefore, the inoculation dose was 10^6 CFU/g. Inoculation was effected using a 1.0 mL syringe and the dose was injected, sprayed and distributed throughout the whole chicken meat. After inoculation, the chicken was wrapped, labelled and stored at ±4°C, ready for irradiation. Dosage levels for irradiation were: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 kGy. For each dose of irradiation, samples were prepared in triplicate.

Enumeration and determination of the surviving number of *L. monocytogenes* was made as follows:

- Twenty-five grams of chicken was homogenated with the addition of 100 mL sterile 0.1% peptone diluent. Stomacher was used for obtaining the homogenized suspension.
- Decimal dilutions were made from the sample with sterile 0.1% peptone diluent.
- *L. monocytogenes* was determined from direct plating of 100 µL suspension on blood agar base media (OXOID CM 55) and plated in duplicate.
- The colonies on agar plates were examined, confirmed and counted. The culture colonies' identities were confirmed by morphology, Gram stain (+) and catalase reaction (+), and motility test (+).

The freeze-dried culture of *C. jejuni* obtained from a local isolate was firstly grown on nutrient broth (with 5% blood), *Campylobacter* blood free selective agar base (OXOID CM 739) or *Campylobacter* agar base (OXOID CM 689), for 48 h at 37°C. Incubation was carried out using an anaerobic jar with 5% oxygen, 10% carbon dioxide, 80% nitrogen and hydrogen (microaerophilic). The purity check of the isolate was done by growing the isolate on blood agar with 5% defibrinated sheep blood. For preparing the inoculum of

C. jejuni, its growth curve in the nutrient broth was first determined. The growth curve (log phase) of *C. jejuni* in nutrient broth was made by enumerating the bacterial concentration on blood agar plates and measuring the optical density at 550 nm of the culture broth. The desired concentration, 10^8 CFU/mL of *C. jejuni*, was used as inoculum. Prior to inoculation, Yunan chicken was cut into pieces and made into 100 g packages. The chicken was then wrapped with aluminum foil and placed in a sterile HDPE bag. The chicken packages were then autoclaved to eliminate all the microorganisms. Chicken inoculations with *C. jejuni* were carried out in a biohazard class II cabinet. Each package (100 g) was inoculated with 1 mL of culture broth containing approximately 10^8 CFU of *C. jejuni.* Therefore, the inoculation dose was 10^6 CFU/g. Inoculation was carried out using a 1.0 mL syringe and the dose injected, sprayed and distributed throughout the chicken meat. After inoculation, the chicken was wrapped, labelled and stored at ±4°C, ready for irradiation with doses of 0.1, 0.2, 0.3 and 0.4 kGy, respectively. For each dose of irradiation, samples were prepared in triplicate.

Enumeration and determination of the number of surviving *C. jejuni* was made as follows:

- Twenty-five grams of chicken was homogenated with the addition of 100 mL sterile nutrient broth. Stomacher was used for obtaining the homogenized suspension.
- Decimal dilutions were made from the sample with the nutrient broth as a diluter.
- *C. jejuni* was determined from direct plating of 100 µL suspension on blood agar base media and plated in duplicate. Incubation was carried out by using an anaerobic jar with 5% oxygen, 10% carbon dioxide, 80% nitrogen and hydrogen.
- The colonies on agar plates were examined, confirmed and counted. The culture colonies identities were confirmed by morphology, Gram stain (–) and catalase reaction (+), oxidase (+) and motility test (+).

In addition, the MPN technique was used for estimating the number of microorganisms *L. monocytogenes* and *C. jejuni* in the chicken sample [14]. Decimal dilutions in LEBB (broth tubes) for *L. monocytogenes* and in nutrient broth (broth tubes) for *C. jejuni,* respectively, were used to recover injured organisms. The manipulation and dilution of samples in LEBB for the MPN method was essential to the procedure used in colony counts. Three tubes were planted for each dilution used. The generally accepted time allowable from preparing the sample homogenate to planting in the last tube was 20 min. Incubation was carried out for 48 h at 37°C for estimating the number of

L. monocytogenes. For *C. jejuni*, incubation was effected by using an anaerobic jar with 5% oxygen, 10% carbon dioxide, 80% nitrogen and hydrogen (micro-aerophilic) for 48 h at 37°C. MPN tables were used for determining MPN estimates for the three tube series.

A flow diagram for counting *L. monocytogenes*/*C. jejuni* [14] in Yunan chicken is illustrated in Fig. 1.

The determination of the D_{10} values (the dose required to inactivate 90% of a population) for some pathogenic bacteria was calculated by plotting ln (N/N_0) against dose (D) according to the equation: $D_{10} = D/(\ln N - \ln N_0)$ where N_0 is the initial number of microorganisms and N is the number of microorganisms surviving the radiation dose [17].

Physicochemical characteristics analysis

The following physicochemical characteristics of the prepared meals, soups, snacks and Yunan chicken were measured: water activity [18, 19], moisture content (%), pH and total fat content (%) and carbohydrate content (%) [20]. Protein content (%) was determined using a combined method of Lowry and biuret reaction [21]. Some other chemical parameter analyses such as peroxide value (meq/kg) [22], fatty acids [23] and amino acid [24] contents (%), vitamin B_1 (ppm) [25, 26], ash and salt contents (%) [27] were only carried out for the Yunan chicken.

Design and analysis of experiments were undertaken statistically according to Steel and Torrie [28].

The nutritive values of raw materials used for each type of soup and snack were calculated on the basis of the quantity of each of the materials according to the following formula:

Nutritive value/serving size/pouch = (weight of material × nutritive value)/100 [29].

2.2.5.3. Sensorial evaluation

Testing of sensory attributes was done by 10 experienced panellists using a 5 point hedonic scale where 5 = excellent, 4 = good, 3 = fair, 2 = poor and 1 = extremely poor [30].

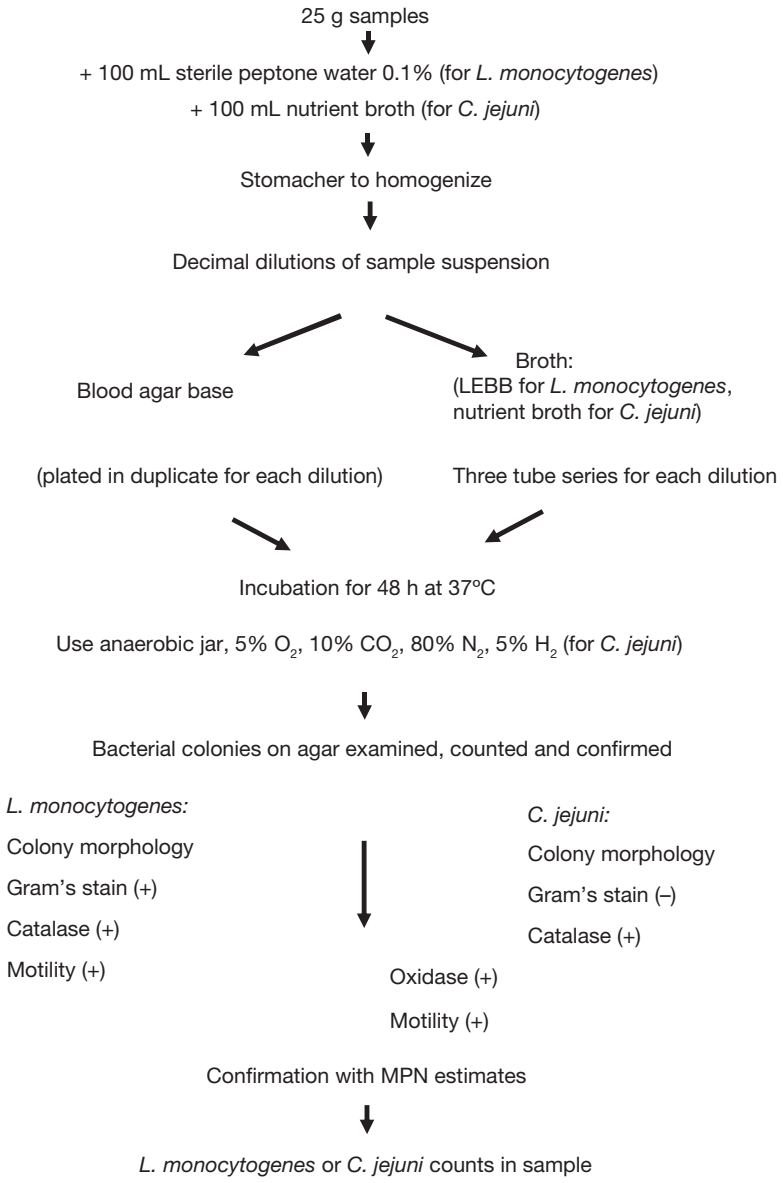

FIG. 1. Flow diagram of procedure for counting L. monocytogenes/C. jejuni *in Yunan chicken.*

3. RESULTS AND DISCUSSION

3.1. Microbiological assessments

3.1.1. Soups

Microbiological assessments (TBC and TMYC) are summarized in Table 1.

The results from Table 2 show that most raw materials used for laboratory made soups, in particular beef and oxtail, contained high levels of bacteria. However, *Staphylococcus* spp. and *E. coli* were not detected in any of the tested samples. Contamination of the ingredients was high in some of them despite the fact that strict selection was done.

The results showed that 5 kGy eliminated all bacteria, moulds and yeasts in black soup. This dose was less effective in the other soups studied; however, all of them presented a very low contamination. Pathogenic bacteria such as *E. coli*, coliforms, *Salmonella* spp., *Staphylococcus* spp. and *Clostridium* spp. were absent from both non-irradiated and irradiated soups.

TABLE 1. EFFECT OF GAMMA IRRADIATION ON MICROBIAL LOAD[a] OF SOUPS AT THE BEGINNING OF STORAGE

Parameter	Irradiation dose (kGy)	Type of soup			
		Black	Oxtail	Chicken vegetable	Chicken sweetcorn
TBC (CFU/g)	0	3.7×10^3	4.6×10^3	7.6×10^3	6.1×10^3
	1	8.5×10^2	2.4×10^2	2.9×10^3	1.5×10^3
	3	1.4×10^2	1.7×10^2	1.4×10^2	1.6×10^2
	5	0	6.0×10^1	7.0×10^1	4.0×10^1
	7	0	0	1.8×10^1	4.0×10^2
TMYC (CFU/g)	0	1.9×10^3	3.0×10^3	4.1×10^3	3.4×10^3
	1	1.0×10^2	1.5×10^2	2.7×10^3	1.4×10^3
	3	0	1.3×10^2	1.5×10^2	1.2×10^3
	5	0	5.0×10^1	6.0×10^1	5.0×10^1
	7	0	0	2.0×10^0	0

[a] Average of three replications.

TABLE 2. MICROBIAL LOAD OF WATER AND RAW MATERIALS AT EACH STEP IN PREPARATION OF LABORATORY SOUPS BEFORE IRRADIATION

Samples	TBC (CFU/g)	TMYC (CFU/g)	*S. aureus* (CFU/g)	*E. coli* (MPN/g)
Tap water (not potable)	1.6×10^3	3.0×10^2	0	0
Cooked water (potable)	2.6×10^2	0	0	0
Raw beef	8.5×10^7	0	0	0
Raw oxtail	5.3×10^7	0	0	0
Raw chicken	2.1×10^3	0	0	0
Raw seasonings for black soup	1.5×10^7	3.9×10^3	0	0
Raw seasonings for oxtail soup	1.1×10^6	0	0	0
Raw seasonings for chicken vegetable soup	5.1×10^4	3.6×10^4	0	0
Raw seasonings for sweetcorn soup	8.5×10^3	1.0×10^3	0	0
Raw mixed vegetables for chicken vegetable soup	1.0×10^3	3.0×10^3	0	0
Raw mixed vegetables for sweetcorn soup	1.9×10^3	1.2×10^3	0	0
Meat broth for black soup	0	0	0	0
Broth for oxtail soup	0	0	0	0
Cooked seasonings for black soup	8.0×10^4	0	0	0
Cooked seasonings for oxtail soup	6.0×10^3	0	0	0
Black soup after cooking	3.6×10^3	0	0	0
Oxtail soup after cooking	5.6×10^3	3.6×10^3	0	0
Chicken vegetable soup after cooking	7.7×10^3	1.2×10^3	0	0
Chicken sweetcorn soup after cooking	1.4×10^3	1.1×10^3	0	0

Warm soups poured into a laminate pouch of PET 12 μm/LDPE as adh. 2 μm/Al foil 7 μm/LDPE as adh./LLDPE 50 μm followed by vacuum sealing gave better results than cold soups without vacuum treatment (preliminary work data are not shown). Packages found during storage which showed damage such as leakage, peeled laminates and swelling were separated and investigated microbiologically, then discarded. The results show that most of the accidents were caused by mishandling during the preparation steps and the growth of *Staphylococcus* spp. found in the swollen packages. The soups were still acceptable from the microbiological viewpoint, but they were not accepted by the panellists owing to their general appearance.

Tables 3 and 4 show that all irradiated samples presented a low microbial load and no pathogenic microorganisms were found except in two samples treated with 1 kGy. Some control samples were positive to coliforms, *S. aureus* and *E. coli.*

3.1.2. Precooked snacks

Table 5 shows the results on microbiological quality of raw mixed vegetables and seasoning used in the preparation of spring roll, rissole and croquette.

Results of the microbial assessment of spring roll are presented in Table 6. On the basis of the microbiological results, this item could be stored at 5 ± 2°C for about 3 months after irradiation with 7 kGy. However, from the sensory evaluation point of view, this product was acceptable only up to 1 month of storage. Pathogenic bacteria such as *E. coli,* coliforms, *Pseudomonas* spp. and *C. perfringens* were not found in any of the samples. After 1 month of storage, the filling of non-irradiated and irradiated (up to 5 kGy) spring rolls became moist and produced some gases. A strong unpleasant odour escaped from the package through tiny pinholes in the sealed area during the storage period. This was probably a result of fermentation due to a biochemical process in the bamboo shoot, which was used as filler material during the preparation of the spring roll. From the obtained results, irradiation with 7 kGy could maintain the quality of the spring rolls vacuum packed in the laminated pouch for 1 month at 5 ± 2°C.

Tables 7 and 8 show that the most effective radiation dose was 7 kGy for both types of snack, i.e. rissole and croquette before and after storage of up to 3 months at refrigeration temperature. Doses of 5 kGy and temperatures 5 ± 2°C can keep the product in good microbiological condition at least for one month. Pathogenic bacteria, i.e. *E. coli,* coliforms, *Pseudomonas* spp. and *C. perfringens*, were negative in all tested samples.

TABLE 3. MICROBIOLOGICAL ASSESSMENTS[a] OF IRRADIATED SOUPS PREPARED IN THE LABORATORY AND PACKED IN VACUUM SEALED LAMINATE POUCHES BEFORE AND AFTER STORAGE AT 5 ± 2°C FOR 3 MONTHS

Parameter	Radiation dose (kGy)	Type of soup and storage time			
		Black		Oxtail	
		0 months	3 months	0 months	3 months
TBC (CFU/g)	0	10	1.3×10^3	10^3	$> 10^6$
	1	0	0	10^3	4.0×10
	3	0	0	0	0
	5	0	0	0	0
	7	0		0	0
TMYC (CFU/g)	0	0	3.8×10^3	0	0
	1	0	0	0	0
	3	0	0	0	0
	5	0	0	0	0
	7	0	0	0	0
Coliforms (MPN/g)	0	15	Negative	Negative	Negative
	1	10	Negative	Negative	Negative
	3	Negative	Negative	Negative	Negative
	5	Negative	Negative	Negative	Negative
	7	Negative	Negative	Negative	Negative
S. aureus (CFU/g)	0	40	0	0	0
	1	0	0	0	0
	3	0	0	20	0
	5	0	0	0	0
	7	0	0	0	0

[a] Average of three replications.

TABLE 4. MICROBIOLOGICAL ASSESSMENTS[a] OF IRRADIATED SOUPS PREPARED IN THE LABORATORY AND PACKED IN VACUUM SEALED LAMINATE POUCHES BEFORE AND AFTER STORAGE AT 5 ± 2°C FOR 3 MONTHS

Parameter	Radiation dose (kGy)	Type of soup and storage time			
		Chicken vegetable		Chicken sweetcorn	
		0 months	3 months	0 months	3 months
TBC (CFU/g)	0	3.3×10^4	1.7×10^5	1.0×10^6	2.5×10^5
	1	1.2×10^4	1.3×10^2	5.0×10^5	2.0×10^2
	3	5.4×10^2	0	1.1×10^4	0
	5	0	0	0	0
	7	0		0	0
TMYC (CFU/g)	0	0	3.3×10^3	0	2.4×10^3
	1	0	0	0	0
	3	0	0	0	0
	5	0	0	0	0
	7	0	0	0	0
Coliforms (MPN/g)	0	Negative	1.1×10^2	Negative	Negative
	1	Negative	Negative	Negative	Negative
	3	Negative	Negative	Negative	Negative
	5	Negative	Negative	Negative	Negative
	7	Negative	Negative	Negative	Negative
E. coli (MPN/g)	0	Negative	Negative	1.1×10^2	Negative
	1	Negative	Negative	8.0×10^1	Negative
	3	Negative	Negative	Negative	Negative
	5	Negative	Negative	Negative	Negative
	7	Negative	Negative	Negative	Negative

[a] Average of three replications.

TABLE 5. MICROBIAL LOAD[a] OF RAW MIXED VEGETABLES AND UNCOOKED SEASONINGS OF SPRING ROLL, RISSOLE AND CROQUETTE

Microbial load[a]	Type of product				
	Raw mixed vegetables	Spring roll filler	Rissole filler	Croquette filler	Bread crumbs
TBC (CFU/g)	1.2×10^4	1.9×10^3	3.3×10^4	3.1×10^4	1.7×10^3
TMYC (CFU/g)	1.1×10^5	1.3×10^5	1.2×10^4	1.3×10^3	1.2×10^3
E. coli (MPN/g)	Negative	Negative	Negative	Negative	Negative
Coliforms (MPN/g)	Negative	Negative	Negative	Negative	Negative
Pseudomonas spp. (CFU/g)	0	0	0	0	0
Staphylococcus spp. (CFU/g)	1.2×10^2	0.5×10^2	2.3×10^2	1.1×10^2	Negative
C. perfringens (MPN/g)	Negative	Negative	Negative	Negative	Negative

[a] Average of three replications.

TABLE 6. MICROBIAL LOAD[a] OF VACUUM PACKED NON-IRRADIATED AND IRRADIATED SPRING ROLL STORED UP TO 3 MONTHS AT 5 ± 2°C

Microbial load[a]	Storage time (months)	Irradiation dose (kGy)			
		0	3	5	7
TBC (CFU/g)	0	2.3×10^3	0	0	0
	1	1.6×10^4	0	0	0
	3	$>10^3$	4.2×10^3	4.5×10^3	0
TMYC (CFU/g)	0	0	0	0	0
	1	1.4×10^3	1.2×10^3	0	0
	3	$>10^3$	4.2×10^2	2.8×10^2	0
Staphylococcus spp. (CFU/g)	0	0	0	0	0
	1	0	0	0	0
	3	$>10^3$	10^2	10	0

[a] Average of three replications.

TABLE 7. MICROBIAL LOAD[a] OF VACUUM PACKED NON-IRRADIATED AND IRRADIATED RISSOLE STORED UP TO 3 MONTHS AT 5 ± 2°C

Microbial load[a]	Storage time (month)	Irradiation dose (kGy)			
		0	3	5	7
TBC (CFU/g)	0	2.7×10^3	0	0	0
	1	4.0×10^3	0	0	0
	3	$>10^3$	4.0×10^2	2.3×10^1	0
TMYC (CFU/g)	0	3.6×10^3	0	0	0
	1	3.9×10^1	1.4×10^1	0	0
	3	$>10^3$	4.1×10^1	1.2×10^1	0
Staphylococcus spp. (CFU/g)	0	0	0	0	0
	1	0	10^2	10	0
	3	$>10^3$	0	0	0

[a] Average of three replications.

TABLE 8. MICROBIAL LOAD[a] OF VACUUM PACKED NON-IRRADIATED AND IRRADIATED CROQUETTE STORED UP TO 3 MONTHS AT 5 ± 2°C

Microbial load[a]	Storage time (month)	Irradiation dose (kGy)			
		0	3	5	7
TBC (CFU/g)	0	9.45×10^2	6.05×10^2	0	0
	1	1.38×10^3	4.37×10^2	0	0
	3	6.37×10^3	3.35×10^2	0	0
TMYC (CFU/g)	0	2.45×10^2	1.71×10^2	0	0
	1	1.56×10^3	1.60×10^2	0	0
	3	2.54×10^3	1.40×10^2	0	0
Staphylococcus spp. (CFU/g)	0	0	$<10^2$	0	0
	1	0	0	0	0
	3	$>10^3$	0	0	0

[a] Average of three replications.

3.1.3. Yunan chicken

The control samples presented a high level of contamination since the beginning of the storage period. Priadi et al. [32] concluded that the microbiological safety and quality of chicken and chicken products collected from the local market were poor and potential sources of foodborne disease outbreak in Indonesia. Ashari et al. [33] came to a similar conclusion for meatball consumption, regarding its safety and convenience. Table 9 summarizes the results of TBC (CFU/g) on commercially prepared Yunan chicken. It shows that irradiation at doses of 3 and 5 kGy was sufficient to eliminate total bacteria in the samples. The TBC (CFU/g) in control samples increased by 3 log cycles after 6 months of storage. Neither *E. coli* nor coliforms were found in the non-irradiated and irradiated samples during storage. The absence of *E. coli* in the samples may be viewed as an indication of good processing steps in the industry or less possibility to grow at the storage temperature. Likewise, other types of Gram negative bacteria such as *Pseudomonas* spp., *Vibrio* spp., and Gram positive bacteria such as *Listeria* spp., are the most critical microorganisms in foodstuffs stored at 5 ± 2°C [34]. Generally, coliforms are not good sanitary indicators for poultry products because other bacteria such as *Salmonellae* may exist in a flock prior to slaughter, and thus a positive faecal coliform test may be unrelated to post-slaughter contamination [35, 36].

Determination of D_{10} values for *S. typhimurium*, *P. aeruginosa* and *E. coli* O157 were 0.28, 0.17 and 0.12 kGy, respectively. If the Yunan chicken is contaminated with those three types of bacteria, the irradiation doses applied should not be less than their *D* values. The D_{10} value for *L. monocytogenes* was 0.66 kGy and for *C. jejuni* it was 0.09 kGy. It seems that the values of the two types of bacteria in Yunan chicken were lower than those in fresh chicken meat. This was probably due to the presence of some natural food additives such as spices and salt added in making Yunan chicken [37].

TABLE 9. MICROBIAL LOAD OF NON-IRRADIATED AND IRRADIATED YUNAN CHICKEN

Irradiation dose (kGy)	TBC (CFU/g) Storage time (weeks)			
	0	3	6	9
0	1.2×10^6	5.9×10^8	9.4×10^9	7.5×10^7
3	0	0	0	0
5	0	0	0	0

3.2. Physicochemical characteristics analysis

3.2.1. Soups

Irradiation (up to 7 kGy) did not affect the pH (6–7) of the soup studied. However, fat and protein contents were affected by irradiation (5 and 7 kGy) and storage time. The moisture content of the soups was in the range 70–87%. The carbohydrate content of the chicken soups varied between 27 and 35%. Fat content was between 10 and 32% and protein content was between 13 and 25%. These values were higher than those for cream soups determined by the Indonesia National Standard [38], and also the carbohydrate contents were higher than the USDA Nutrient Database for Standard Reference [27]. A slight reduction was found in the fat and protein contents of certain irradiated soups during storage. The selected storage temperatures were not effective enough to maintain the stability of either the fat or the protein contents in the semi-liquid samples.

The purpose of calculating the nutritive values/serving size/pouch of the soups was to assess the nutrition factor of each raw material prior to use, based on some essential food components (Table 10).

TABLE 10. PROXIMATE NUTRITIVE VALUE OF THE SOUPS STUDIED

Nutrient (unit)	Nutritive value/serving size/pouch of soup (weight of material × nutritive value)/100			
	Black 300 g/pouch	Oxtail 300 g/pouch	Chicken vegetable 300 g/pouch	Chicken sweetcorn 250 g/pouch
Energy (cal)	796	466	674	659
Protein (g)	26.6	19.5	32.8	32.3
Fat (g)	74.4	40.7	46.1	51.3
Carbohydrate (g)	54.4	3.99	26.2	17.8
Calcium (mg)	32.2	29.4	87.6	45.5
Phosphorus (mg)	249	190	493	353
Iron (mg)	4.17	3.46	5.47	3.35
Vitamin A (IU)	30.045	15.128	4.702	2.841
Vitamin B_1 (mg)	0.136	0.299	0.374	0.252
Vitamin C (mg)	5.77	3.98	24.2	4.87

3.2.2. Precooked snacks

The nutritive values of precooked snacks are shown in Table 11.

The results of water activity measurement of either non-irradiated or irradiated spring roll samples were within the range of 0.80–0.90. Some other quality parameters, i.e. pH value, moisture and protein contents in the spring roll before and after irradiation at doses of 5–7 kGy without storage indicate that the samples were stable after the treatment (pH4.6–4.8, moisture content 72–73% and protein content 3.64–3.76%), carbohydrate content slightly increased at the dose of 7 kGy, but fat content slightly reduced at the dose of 5 kGy (from 3.9% to 2.99%).

The results of physicochemical characteristics of rissole and croquette (Tables 12 and 13) showed that pH values and fat content of the non-irradiated and irradiated samples decreased during the 3 months storage. It was probably caused by the biochemical degradation of some macrocomponents within the products. On the basis of the overall results, rissole and croquette showed more promising applications of irradiation than spring rolls.

TABLE 11. PROXIMATE NUTRITIVE VALUE OF PRECOOKED SNACKS MADE IN HOME INDUSTRY

Nutrient (unit)	Nutritive value/serving size/pouch of snack (weight of material × nutritive value)/100		
	Spring roll/ 100 g	Rissole/ 100 g	Croquette/ 100 g
Energy (cal)	141.80	284.68	131
Protein (g)	6.46	15.11	5.15
Fat (g)	4.76	16.99	7.39
Carbohydrate (g)	13.67	12.11	11.2
Calcium (mg)	27.39	236.76	21
Phosphorus (mg)	85.45	244.83	81.7
Iron (mg)	1.47	2.228	1
Vitamin A (IU)	156.91	742.77	206.3
Vitamin B_1 (mg)	0.068	0.165	0.086
Vitamin C (mg)	3.49	1.219	9.45

TABLE 12. RESULTS OF PHYSICOCHEMICAL ANALYSIS[a] OF RISSOLE BEFORE AND AFTER IRRADIATION AT DOSES UP TO 7 kGy AND STORED AT 5 ± 2°C

Parameter	Storage time (months)	Irradiation dose (kGy)			
		0	3	5	7
Water activity	0	0.81 ± 0.00	0.85 ± 0.04	0.87 ± 0.01	0.92 ± 0.02
	3	0.86 ± 0.01	0.86 ± 0.00	0.80 ± 0.00	0.90 ± 0.01
pH	0	7.60 ± 0.02	7.35 ± 0.02	7.45 ± 0.04	7.45 ± 0.01
	3	4.21 ± 0.02	4.40 ± 0.02	5.30 ± 0.08	5.90 ± 0.70
Moisture content (%)	0	38.04 ± 0.29	38.21 ± 0.22	37.96 ± 0.02	38.78 ± 0.21
	3	38.75 ± 1.00	38.31 ± 0.89	41.82 ± 0.19	40.32 ± 0.75
Carbohydrate (%)	0	16.56 ± 0.48	17.47 ± 0.33	16.66 ± 0.31	16.52 ± 0.46
	3	15.92 ± 1.71	17.35 ± 1.54	16.00 ± 0.44	15.65 ± 0.43
Fat (%)	0	6.50 ± 0.36	5.14 ± 0.25	6.86 ± 1.01	7.35 ± 1.11
	3	5.16 ± 0.12	4.60 ± 0.19	4.07 ± 0.99	5.22 ± 0.21
Protein (%)	0	0.13 ± 0.00	0.13 ± 0.01	0.13 ± 0.00	0.12 ± 0.01
	3	0.08 ± 0.00	0.09 ± 0.00	0.10 ± 0.01	0.10 ± 0.00

[a] Average of three replications.

3.2.3. Yunan chicken

There was no important effect of irradiation on the water activity (0.92–0.95), pH (6.0–6.4) and moisture content (59–60%) of Yunan chicken.

Irradiation treatment did not affect the fat content, which has also been demonstrated by other authors [6, 9]. The effect of ionizing radiation on the fatty acids in poultry meat and its products strongly depends on the presence of unsaturated fatty acids [39–41]. The results from irradiated Yunan chicken during nine weeks of storage indicated that the most radiosensitive fatty acid component to irradiation at 5 kGy was palmitic acid (C16:0) (from 21% to 17%), followed by oleic acid (C18:1) (from 33% to 31%) and linoleic acid (from 19% to 15%). The percentage of palmitoleic (C16:1) (1.02–1.11%) and arachidic (C20:2) (0.15%) acids were relatively stable after the same treatment.

TABLE 13. PHYSICOCHEMICAL CHARACTERISTICS[a] OF CROQUETTE BEFORE AND AFTER IRRADIATION OF UP TO 7 kGy DURING STORAGE AT 5 ± 2°C

Parameter	Storage time (months)	Irradiation dose (kGy)			
		0	3	5	7
Water activity	0	0.89 ± 0.00	0.87 ± 0.00	0.89 ± 0.00	0.90 ± 0.01
	3	0.88 ± 0.00	0.88 ± 0.01	0.86 ± 0.00	0.85 ± 0.00
pH	0	6.63 ± 0.01	6.64 ± 0.01	6.65 ± 0.00	6.66 ± 0.01
	3	3.85 ± 0.02	4.30 ± 0.03	4.48 ± 0.02	5.88 ± 0.09
Moisture content (%)	0	36.37 ± 1.33	39.46 ± 0.54	39.09 ± 0.15	39.92 ± 0.22
	3	40.34 ± 0.15	40.58 ± 0.22	41.82 ± 0.18	39.70 ± 0.39
Carbohydrate (%)	0	17.79 ± 0.17	17.73 ± 0.06	17.56 ± 0.06	17.45 ± 0.07
	3	16.50 ± 0.30	16.84 ± 1.26	16.59 ± 1.13	15.92 ± 0.12
Fat (%)	0	10.09 ± 0.82	7.31 ± 1.15	8.06 ± 0.07	9.68 ± 0.66
	3	6.45 ± 0.35	5.30 ± 0.25	7.46 ± 0.25	9.23 ± 1.03
Protein (%)	0	0.10 ± 0.01	0.11 ± 0.01	0.12 ± 0.00	0.12 ± 0.11
	3	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.01

[a] Average of three replications.

Table 14 summarizes data on the measurement of peroxide value in irradiated Yunan chicken. The values showed an increase with higher doses. However, the values decreased during the storage time. Interaction between irradiation and storage time could significantly reduce the peroxide value in the samples. Unsaturated acyl lipids as well as acyl lipid constituents, which include oleic, linoleic and linolenic acids, have one or more allyl groups within the fatty acid molecules which are readily oxidized to hydroperoxide, and after subsequent degradation induced by radiolysis might yield a great number of other compounds. The degradation process either induced by auto-oxidation or by lipid peroxidation of oleic, linoleic and linolenic acids might liberate some of the numerous volatile and non-volatile compounds such as aldehydes and ketones which might affect the aroma and flavour of the products. Post-irradiation effects such as auto-oxidation of unsaturated acyl lipids in irradiated Yunan chicken could be retarded and suppressed through exclusion of oxygen within the package prior to process, irradiation under cryogenic conditions and storage of the irradiated products at low temperature (5 ± 2°C).

TABLE 14. EFFECT OF GAMMA IRRADIATION ON PEROXIDE VALUE (mek/kg) OF NON-IRRADIATED AND IRRADIATED YUNAN CHICKEN STORED AT 5 ± 2°C

Irradiation dose (kGy)	Peroxide value (mek/kg)			
	Storage time (weeks)			
	0	3	6	9
0	237.72 ± 0.04a*	65.26 ± 0.32b	33.32 ± 0.98c	36.92 ± 0.34d
3	310.41 ± 0.03e	66.56 ± 0.89b	23.92 ± 0.44f	23.98 ± 0.64f
5	586.04 ± 0.94g	98.00 ± 0.64h	96.91 ± 0.83h	57.54 ± 0.16i

* Means for each character followed by the same letter are not significantly different at $p < 0.05$.

The determination of protein and major amino acid contents indicated that irradiation with doses up to 5 kGy did not have any significant effect on protein content (33–34%) and neither did the major amino acids in the samples. These results are supported by several authors [41]. It is reported that in most experiments there were no significant changes in amino acid composition in the dose range below 50 kGy. If there was any effect of irradiation, it was within the range of biological variability. Likewise, the storage time and the interaction between irradiation and storage time could slightly influence the protein content as well as in the analysis of amino acids in the analysed samples.

Chicken meat contains vitamin B_1 which is very sensitive to radiation exposure. It cannot be protected solely by the exclusion of oxygen from the package, but also requires the temperature during the radiation process to be maintained. Yunan chicken was placed in vacuum packed laminate pouches and irradiated under cryogenic conditions at doses of up to 5 kGy and the vitamin was slowly reduced by about 20–50% both before (from 0.11 mg/100 g to 0.04 mg/100 g edible portion) and after storage (from 0.13 mg/100 g to 0.06 mg/100 g edible portion). Severe damage in vitamin B_1 might occur if the radiation processing of Yunan chicken is conducted in the presence of oxygen and at ambient temperature.

Previous studies on irradiated minerals and trace elements indicated that irradiation does not affect some micronutrients and there is no evidence that the bioavailability of these elements might be adversely affected by irradiation [31, 40]. The effect of ionizing radiation on Yunan chicken resulted in some fluctuating values in ash content (3–4%) and this is probably caused by the

processing step and types of seasoning used in its preparation. Ash content in the processed meat was about 0.7–1.3% [42]. The increase in salt content in irradiated Yunan chicken during storage was probably due to greater salt absorption within the product with increasing length of storage. Unfortunately, the quantity of salt added in the Yunan chicken during preparation was not well documented by the food industry.

3.3. Sensory evaluation

3.3.1. Soups

The results obtained in sensory evaluations, i.e. appearance, aroma, taste and texture/consistency of the products, indicated that the values of each attribute remained high (3–4), but non-irradiated soups were mostly rejected (1–2) after 1 month of storage at 5 ± 2°C.

3.3.2. Precooked snacks

The results from treatment of spring roll indicate that irradiation at 7 kGy followed by 1 month's storage at 5 ± 2°C could maintain the score of sensory attributes, i.e. general appearance (3.8), colour (3.5), texture (3.3), aroma (2.8) and taste (2.4). However, after 1 month, all samples were rejected by the panellists. Sensory tests of rissole and croquette showed that irradiation at doses of 5 and 7 kGy and storage times of up to 3 months at 5 ± 2°C could significantly maintain general appearance (3.3), aroma (3.8) and taste (3.3) of rissole, but a dose of 5 kGy gave better sensory results for colour (4.0) and taste (3.0) than one of 7 kGy in the case of croquette under the same storage conditions. The laminate packaging material used and the packing technique may play an important role in maintaining the quality of these products during storage, but some samples remained attached to the inner layer and needed to be slowly removed prior to analysis. It is suggested that an intermediate package, as well as PET trays or HDPE film sheet as direct contact packing material to the product, is needed. A polypropylene tray is not suitable for such a purpose because it is not able to withstand irradiation at –79°C.

3.3.3. Yunan chicken

On the basis of the results of the overall sensory evaluation of irradiated Yunan chicken at doses of up to 5 kGy and storage periods of up to 9 months, the product was still accepted by the panellists within the score range of 3 to 4. There was no effect on either flavour or aroma of fatty acid changes induced by radiation.

4. CONCLUSIONS AND RECOMMENDATIONS

It can be concluded from the results that gamma irradiation at pasteurization doses of 5–7 kGy in combination with cryogenic conditions (–79°C) could maintain the safety and quality of four types of home made soup packaged in a laminate pouch under vacuum and stored for 3 months at 5 ± 2°C. Irradiation at doses of 5–7 kGy could also extend the shelf life of rissole and croquette for 3 months at the same temperature without significantly affecting their general quality. Gamma irradiation as cold pasteurization at doses of 3 and 5 kGy could maintain the overall quality of Yunan chicken for up to 9 months with storage at 5 ± 2°C.

The use of direct contact as well as a flexible sheet and rigid packing material inside the laminate pouch and the development of a packing technique are needed in order to protect against the damage caused by vacuum treatment.

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Annex I

RAW MATERIALS, INGREDIENTS AND QUANTITIES FOR MAKING BLACK, OXTAIL, CHICKEN VEGETABLE AND CHICKEN SWEETCORN SOUPS IN THE LABORATORY

Materials/ ingredients	Soup			
	Black Quantities for 20 packages @ 300 g (1)	Oxtail Quantities for 20 packages @ 300 g (2)	Chicken vegetable Quantities for 18 packages @ 200 g (3)	Chicken sweetcorn Quantities for 25 packages @ 250 g (4)
Beef	2.5 kg			
Oxtail		2 kg		
Chicken			3 kg	3 kg
P. edule	275 g			
Shallot	175 g	70 g	105 g	
Garlic	35 g	35 g	30 g	
Roasted coriander	25 g			
Roasted candlenut	100 g			
Seedless red chilli	50 g			
Ginger	10 cm			
Aromatic lemon leaf	5 g			
Roasted fish paste	6 g			
Turmeric	10 cm			
Ginger root	10 cm			
Lemon grass	100 g			
Kaempferia galanga	10 cm			
Salt	20 g	25 g	25 g	75 g
Palm sugar	15 g			
Bay leaf	10 g			
Palm oil	1 L	500 mL		

RAW MATERIALS, INGREDIENTS AND QUANTITIES FOR MAKING BLACK, OXTAIL, CHICKEN VEGETABLE AND CHICKEN SWEETCORN SOUPS IN THE LABORATORY

Materials/ ingredients	Soup			
	Black Quantities for 20 packages @ 300 g (1)	Oxtail Quantities for 20 packages @ 300 g (2)	Chicken vegetable Quantities for 18 packages @ 200 g (3)	Chicken sweetcorn Quantities for 25 packages @ 250 g (4)
Water	5 L	5 l	3 l	3 l
Onion		50 g		
Ground nutmeg		20 g	12.5 g	40 g
Cloves		2 g		
Ground white pepper		25 g	20 g	
Onion leaf		100 g	200 g	
Celery		75 g	150 g	
Margarine		30 g	75 g	
Sugar (cane)			25 g	
Young sweetcorn				5 kg
Chicken sausage				1100 g
Carrot			450 g	350 g
Eggs				400 g
Corn starch				
Green beans			350 g	
Broccoli			400 g	
Frozen sugar peas			500 g	

Annex II

RAW MATERIALS, INGREDIENTS AND QUANTITIES FOR MAKING SPRING ROLLS, RISSOLES AND CROQUETTES

	Spring roll and filler	Rissole filler	Croquette filler
Ready prepared spring roll sheets	200 g		
Margarine		30 g	30 g
Minced beef/ chicken	250 g	50 g	150 g
Peeled shrimp	100 g		
Young bamboo shoot	300 g		
Wheat flour		16 g	
Chicken broth		100 mL	100 mL
Shallot		20 g	20 g
Garlic	120 g	20 g	
Salt	16 g		
Sago	5 g		
Sweet soya sauce	2.5 g		
Green beans	15 g	50 g	
Carrot			100 g
Cheese		100 g	
Ground peppers, salt		5 g	
Eggs		125 g	
Wrapper		Rissole	Croquette
Milk		250 mL	
Eggs		125 g	125 g
Wheat flour		20 g	
Salt		4 g	
Steamed potatoes			500 g
Chicken broth			100 mL
Ground nutmeg			7.5 g
Breadcrumbs		100 g	100 g

PART I: HAZARD, SENSORIAL AND ECONOMIC IMPLICATIONS OF APPLYING THE HAZARD ANALYSIS AND CRITICAL CONTROL POINTS TO IRRADIATED READY-TO-EAT MEALS

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Abstract

The classical methodology of hazard analysis and critical control points focuses on hazards and related implications. A new methodology is suggested here, one that attempts at a systematic simultaneous assessment of safety hazards, sensorial failures and economic risks, and the critical control points necessary for their early detection and/or prevention of their potential outcomes. The new methodology also attempts to combine the three parameters to form a qualitative prioritization of the numerous control points and to screen those that can be cost effective for implementation. This is demonstrated in this paper for a complex product, i.e. radiation sterilized ready-to-eat meals. Hence, a fourth parameter specific to this product — the radiation specific pitfall — is also assessed. The advantages and drawbacks of the combined assessment methodology are described and their overall possible impact is discussed. Finally, the suggested combined assessment and control system for ensuring the safety and quality of food can provide a more structured and critical approach to control identified hazards, compared with that achievable by traditional inspection and quality control procedures. It has the potential to identify areas of concern where failure has not yet been experienced, making it particularly useful for new operations and products thereafter.

1. INTRODUCTION

The classical methodology of hazard analysis and critical control points (HACCP) stems from regulations and liability aspects of production operations and their corresponding commercial products. The classical HACCP does not aim to be, nor does it provide, a means of assessing failure modes that relate mainly to marketing or economic disasters. Nevertheless, it is the potential commercial success that drives the producer to invest in the assessment, detection and prevention of failure modes. Thus, it seems important to harness the HACCP in the drive for commercial success and increased profits, via a new combined HACCP methodology, as discussed here.

In the food industry, the ready-to-eat sector is a fast growing one with failure modes that may be concealed up to a point, in particular the failure modes that correlate to the unskilled consumer and/or food preparer customers. While success in this sector of the market may be highly beneficial, failure may be disastrous for numerous reasons. In this already complex field, radiation processing offers a unique opportunity for pasteurized or sterilized foods.

Nevertheless, the use of irradiation technology to reduce/eliminate bacteria necessitates skilful assessment of the radiation specific failure modes, sensorial in principle, to ensure the anticipated economic success.

2. METHODOLOGY

2.1. Assessment parameters

A systematic assessment of four parameters is discussed. The assessment was carried out at each stage of food processing (Table 1), and in the same order. This systematic approach was found useful to ensure consideration of each cause of failure and the consequent failure mode. Regarding specifically HACCP analysis of a ready-to-eat-meal, the primary concern, as in the classic HACCP, is the concern of hazard implications stemming from the safety and health of the end consumer. In this respect, intermediate customers of the industry (e.g. distributors) and food preparers are much less of a priority.

Next to the primary parameter in the combined HACCP assessment, the safety/health hazard, the second one is the sensory quality of the food. This parameter is crucial for reaching the objective of a viable food business, rather than an anecdotal research food. The third parameter is the economic impact of each and every failure mode of the food, also crucial for becoming a viable food business. These last two parameters ought to indicate that the business aspects of the food are well taken care of, thus leaving a sufficient profit margin to finance an uncompromised care of the hazards per se.

Last but not least, the radiation specific nature of each and every failure cause and failure mode is assessed, aimed at bringing attention to the sensory relevant and business relevant technological differences between radiation processed food and food that is processed only by traditional means.

2.2. Assessment stages

In the following combined multiparameter assessment (Table 1), the stages of food processing aim to cover each distinct operation, 'from farm to fork', from harvesting crops through to assessing the hazard relevant quality of: (1) the ingredients, (2) processing the ingredients, (3) mixing and structuring of the food, (4) heat treatments of the food (baking, frying, cooking), (5) packaging the food, (6) irradiation produced, (7) pasteurization or sterilization, (8) distributing the food, (9) preparing the food for the end consumer, and (10) actual consumption steps.

The special importance of consumer responsibility in preventing hazards and complaints afterwards is further discussed, focusing on the importance of appropriate consumer guidance.

3. RESULTS AND DISCUSSION

3.1. HACCP

The comprehensive combined HACCP chart is exemplified in Table 1.

3.2. A retrospective hazard analysis: The Bamba story

The importance of the comprehensive HACCP shown in Table 1 is demonstrated in the following anecdote. The fatal consequences could have been prevented ab initio if the HACCP had been adequately carried out.

About 15 years ago, a severe epidemic of *Salmonella* affecting children was reported in London. There were two mortalities. A quick investigation revealed that all the sick children were from the orthodox Jewish community. The significance of this was that all the children used to consume special kosher snacks that were all imported from Israel for the sole consumption of that community. Their favorite snack was Bamba, a peanut flavoured puffed corn snack which catered for toddlers and very young children, as it dissolved in the mouth without requiring chewing.

Once the origin of the hazard was identified, all the imported snacks were recalled and immediately sent for bacterial analysis. Not surprisingly, the recalled Bamba snacks were found to be highly contaminated with *Salmonella*. A special investigator was appointed to inspect all Bamba ingredients and equipment, aiming at tracking down the origin of the bacteria.

TABLE 1. THE COMBINED HACCP

Control point	Failure mode/hazard	Preventive control measure	Detectability	Haz.	Sens.	Econ.	Rad. specific
Raw materials: Vegetables							
Bacterial	Bacterial contamination due to irrigation with sewage contaminated water	Irrigation inspection	Microbiological smear testing	✓✓			
Bacterial	Contamination in the field by birds, rodents or insects	Bird, rodent and insect control field programme	Inspection: Microbiological smear testing	✓✓			
Bacterial	Post-harvest contamination due to injuries	Discard of damaged vegetables	Inspection for damaged vegetables		✓✓		
Bacterial	Storage: Mould or bacterial growth due to damp storage conditions	Dry storage/humidity control and moisture control in storage warehouse	Microbiological smear testing	✓✓	✓		
Bacterial	Storage: Mould or bacterial growth due to excessive temperature storage conditions	Temperature control in storage warehouse	Microbiological smear testing	✓✓	✓		
Bacterial	Storage: Contamination by birds, rodents or insects	Bird, rodent and insect control storage programme	Inspection: Microbiological smear testing	✓✓	✓	✓	
Bacterial	Supplier: High microbial loading of unknown source	Selection of suppliers of hygiene practices	Microbiological smear testing	✓✓			

TABLE 1. THE COMBINED HACCP (cont.)

Control point	Failure mode/hazard	Preventive control measure	Detectability	Haz.	Sens.	Econ.	Rad. specific
Biotoxins	Accumulation of biotoxins due to sustained exposure to bacteria and/or moulds	Post-detection: Discard batch	Biotoxins smear testing	✓✓	✓✓	✓✓	
Pesticides	Contamination by pesticides	Post-detection: Discard batch	Pesticide smear testing	✓✓		✓✓	
Physical	Faulty texture, rigidity, stem cut or damaged skin	Harvesting quality protocols, monitoring and sorting	Inspection	✓✓	✓✓	✓✓	✓✓
Physical	Contamination by foreign matter, e.g. stones, metal objects, shells	Inspection, sieving and washing Set threshold for lot discarding	Inspection, sieving and washing	✓✓	✓✓	✓✓	
Physical	High concentration of hard fibres or grainy material	Inspection, sorting Set threshold for batch discard	Inspection, mechanical testing		✓✓		
	Storage: Spoilage by exposure to extreme temperatures	Temperature control in storage warehouse	Microbiological smear testing	✓✓	✓		
Temperature	Excessively high temperature upon harvesting, resulting in damaged skin	Inspection, sorting Set threshold for batch discard	Inspection, mechanical testing		✓✓		
Temperature	Excessively low temperature upon intermediate storage, resulting in damaged skin	Inspection, sorting Set threshold for batch discard	Inspection, mechanical testing		✓✓		

TABLE 1. THE COMBINED HACCP (cont.)

Control point	Failure mode/hazard	Preventive control measure	Detectability	Haz.	Sens.	Econ.	Rad. specific
Raw materials: Spices — all of the above as well as:							
Active material concentration	Variation in concentration of active material, e.g. capsicum, ginger, bacterial growth	Adjust recipe to maintain spice level within set limits	Analysis of spices	✓	✓✓		✓✓
Acidity concentration	Variation in concentration of acid, e.g. lemon, vinegar, bacterial growth	Adjust recipe to maintain spice level within set limits	Analysis of spices	✓	✓✓		✓✓
Fermentation products	Variation in concentration of active material and bacterial growth	Adjust recipe to maintain spice level within set limits	Analysis of spices	✓	✓✓		✓✓
Salt mixtures	Variation in concentration salt and bacterial growth	Adjust recipe to maintain spice level within set limits	Analysis of spices	✓	✓✓		✓✓
Raw materials: Animal products							
Bacterial	Bacterial contamination from burst intestines	Discard parts contaminated beyond permitted level	Inspection and microbiological testing	✓	✓		✓
Bacterial	Bacterial contamination from cuts, bruises and abscesses	Discard parts contaminated beyond permitted level	Inspection and microbiological testing	✓	✓		✓
Bacterial	Bacterial contamination from insect bites and larvae therein	Discard parts contaminated beyond permitted level	Inspection and microbiological testing	✓	✓		✓

TABLE 1. THE COMBINED HACCP (cont.)

Control point	Failure mode/hazard	Preventive control measure	Detectability	Haz.	Sens.	Econ.	Rad. specific
Physical	Bones, teeth, scale and their fragments	Discard parts physically contaminated beyond permitted level	Inspection	✓	✓	✓	
Fat content	Fat degradation during irradiation and production of 'off' odours	Discard parts containing fat beyond a predetermined level	Inspection	✓	✓		✓
Raw materials: Packaging							
Bacterial (pre-packaging)	Contamination by birds, rodents or insects	Bird, rodent and insect control programme	Packaging batch inspection	✓✓	✓✓		
Bacterial (pre-packaging)	Contamination by dirt from machinery and workers	Clean storage environment	Packaging batch inspection	✓✓	✓✓		
Bacterial (post-packaging)	Contamination by penetration of birds, rodents or insects	Bird, rodent and insect control programme	Packaged batch inspection	✓	✓✓	✓✓	✓✓
Bacterial (post-packaging)	Contamination (external) by dirt	Clean storage environment	Packaged batch inspection	✓	✓✓		
Packaging damage	Contamination by penetration of insects and bacteria	Discard damaged packaging materials	Inspection of packaging prior and after packaging	✓	✓✓	✓	✓✓

TABLE 1. THE COMBINED HACCP (cont.)

Control point	Failure mode/hazard	Preventive control measure	Detectability	Haz.	Sens.	Econ.	Rad. specific
Processing: Cutting (or pressing)							
Bacterial	Inclusion of bacteria from contaminated cutting equipment and/or boards	Pre-cleaning and temperature control of cutting equipment Discard contaminated batches	Microbiological inspection of cutting equipment and processed batches	✓	✓✓	✓	✓✓
Bacterial	Inclusion of bacteria from chopped insects or small rodents or their litter	Inspection of pre-processed batches for insects or rodents or litter (Bamba story)	Microbiological/ foreign particle inspection of processed batches	✓	✓✓	✓	✓✓
Bacterial	Bacterial growth due to processing temperature	Temperature control of cutting equipment Discard contaminated batches	Microbiological inspection of processed batches	✓	✓✓	✓	✓✓
Foreign objects	Inclusion of foreign objects from cutting equipment and/or boards	Pre- and post-and processing inspection of cutting equipment Discard suspicious batches	Pre- and post-processing inspection of cutting equipment	✓✓	✓✓	✓✓	
Processing: Mixing							
Bacterial	As above plus inclusion of bacteria from a specific ingredient	Discard any contaminated ingredient	Microbiological inspection of mixed batches	✓	✓✓	✓	✓✓

TABLE 1. THE COMBINED HACCP (cont.)

Control point	Failure mode/hazard	Preventive control measure	Detectability	Haz.	Sens.	Econ.	Rad. specific
Foreign objects	Inclusion of foreign objects from cutting equipment and/or boards	Pre- and post-processing inspection of mixing equipment Discard suspicious batches	Pre- and post-processing inspection of mixing equipment	✓✓	✓✓	✓✓	
Processing: Cooking							
Bacterial	Inclusion of bacteria from cookware or transfer tools	Discard batches contaminated beyond permitted level	Inspection and microbiological testing of cookware and food	✓	✓	✓	✓
Foreign objects	Inclusion of foreign objects from cookware or transfer tools	Pre- and post-cooking inspection of cookware Discard suspicious batches	Pre- and post-processing inspection of cookware	✓✓	✓✓	✓✓	
Overcooking	Spoiled food from overcooking or defective stirring Radiation enhancement of off taste	Discard spoiled batches	Monitor stirring, temperature, time		✓✓	✓✓	✓✓
Texture spoilage	Spoilage of food texture by tools used for transfer or stirring	Discard spoiled batches	Monitor stirring tools and process		✓	✓	

TABLE 1. THE COMBINED HACCP (cont.)

Control point	Failure mode/hazard	Preventive control measure	Detectability	Haz.	Sens.	Econ.	Rad. specific
Processing: Frying							
Bacterial	Inclusion of bacteria from fry-ware or transfer tools	Discard batches contaminated beyond permitted level	Inspection and microbiological testing of fry-ware and food	✓	✓	✓	✓
Foreign objects	Inclusion of foreign objects from fry-ware or transfer tools	Pre- and post-frying inspection of fry-ware Discard suspicious batches	Pre- and post-processing inspection of fry-ware	✓✓	✓✓	✓✓	
Overfrying	Inclusion of spoiled food from overfrying or defective stirring	Discard spoiled batches	Monitor stirring, temperature, time		✓✓	✓✓	
Texture spoilage	Spoilage of food texture by tools used for transfer or stirring	Discard spoiled batches	Monitor stirring tools and process		✓	✓	
Rancidization	Off taste due to use of spoiled oil Radiation enhancement of off taste	Discard spoiled batches	Frying oil packaging QA Laboratory and/or sensory inspection		✓✓	✓✓	✓✓
Rancidization	Off taste due to oil spoilage on overheating Radiation enhancement of off taste	Discard spoiled batches	Monitor temperature, time, stirring		✓✓	✓✓	✓✓

TABLE 1. THE COMBINED HACCP (cont.)

Control point	Failure mode/hazard	Preventive control measure	Detectability	Haz.	Sens.	Econ.	Rad. specific
Oil inclusion	Excessive inclusion of oil in the food due to underheating Radiation enhancement of off taste	Discard spoiled batches	Monitor temperature upon introduction of food		✓✓	✓✓	✓✓
Sticking	Spoilage of food texture by sticking onto fry-ware	Discard spoiled batches	Monitor stirring tools and process		✓✓	✓✓	
Processing: Structuring and stuffing							
Bacterial	Inclusion of bacteria from processing tools	Discard batches contaminated beyond permitted level	Inspection and microbiological testing of tools and food	✓	✓	✓	✓
Foreign objects	Inclusion of foreign objects from processing tools	Pre- and post-processing inspection of tools Discard suspicious batches	Pre- and post-processing inspection of tools	✓✓	✓✓	✓✓	
Internal texture spoilage	Spoilage of inner food texture by processing tools	Discard spoiled batches	Monitor shear forces on food		✓✓	✓✓	
External texture spoilage	Spoilage of envelope food texture by processing tools	Discard spoiled batches	Monitor food structuring operations		✓	✓✓	

TABLE 1. THE COMBINED HACCP (cont.)

Control point	Failure mode/hazard	Preventive control measure	Detectability	Haz.	Sens.	Econ.	Rad. specific
Processing: Packing							
Bacterial	Inclusion of bacteria from packaging equipment	Discard contaminated batches	Pre- and post-packaging inspection of food microbiological level	✓	✓	✓✓	✓✓
Foreign objects	Inclusion of foreign objects from packaging equipment	Discard spoiled batches	Pre- and post-packaging inspection of tools and equipment	✓	✓	✓✓	
External texture spoilage	Spoilage of envelope food texture by packaging equipment	Discard spoiled batches	Pre- and post-packaging inspection of food texture	✓	✓	✓✓	
Storage							
Bacterial	Microbial growth in moist products	Storage of moist products at $5 \pm 2^{o}C$ Limited shelf life	Monitor temperature and food spoilage	✓	✓	✓✓	✓✓
Distribution							
Bacterial	Microbial growth in moist products	Distribution chain of moist products at $5 \pm 2^{o}C$ Limited shelf life	Chill distribution chain $5 \pm 2^{o}C$	✓	✓	✓✓	✓✓

TABLE 1. THE COMBINED HACCP (cont.)

Control point	Failure mode/hazard	Preventive control measure	Detectability	Haz.	Sens.	Econ.	Rad. specific
Bacterial	Distribution of out of date product	Date label and stock rotation control	Date, label and stock rotation control	✓	✓	✓✓	✓✓
Consumer							
Bacterial	Storage abuse of product leading to microbial growth	Clear instructions to the consumer on storage, shelf life and product preparation	Clear instructions to the consumer on storage, shelf life and product preparation	✓	✓	✓✓✓	✓✓
All steps							
Bacterial (personnel)	Contamination due to unsanitary handling practices	Personnel hygiene enforcement Discard contaminated batches	Personnel hygiene inspection	✓✓	✓	✓✓	✓✓
Bacterial (personnel)	Cross-contamination from raw material, germination and growing areas	Enforcement of controlled movement of staff and equipment Discard contaminated batches	Controlled movement of staff and equipment	✓✓	✓	✓✓	✓✓
Bacterial (personnel)	Metal fragments in product	Use of metal detector	Use of metal detector	✓✓	✓✓	✓	✓✓✓

The only ingredient carrying the bacteria was identified as peanut butter imported from South Africa. Here, the contamination source was tracked to the pre-pressed raw peanuts, which were detected as carrying a huge flora of *Salmonella*, among other bacteria. A close inspection of the pile of raw peanuts in the open shed farm storehouse revealed that the piles of peanuts included significant amounts of rodent litter. The above 'detective' story is a mishap chronology, typical of products with inadequate HACCP. In retrospect, it seems rather trivial to carry out proper HACCP for food products. In reality, numerous mishaps similar to the above take place all the time, probably creating insufficient understanding of the trade-off between the HACCP endeavour and cost, on the one hand, and possible mishaps, exertion and damages on the other.

3.3. Pros and cons of carrying out a comprehensive HACCP

It is quite obvious that each HACCP has a price tag. The more comprehensive the HACCP and the consequential tests at numerous control points, the higher this price tag becomes. The pros are obvious: the more comprehensive the HACCP, the smaller the risk of marketing an unsafe food, and facing lawsuits, fines and compensations thereafter. The cons are trivial: the more expensive the HACCP becomes, the bigger the risk that the product will fail, business-wise.

Hence, the crucial question stemming from the above displayed HACCP methodology is how to reach an optimum balance between the value of producing a safe food and the need to keep the costs at a reasonable level and to ensure reasonable profits for the producer and thereby, a sustained business. The rationale leading to this optimum seems straightforward: critical control points should be prioritized for implementation, on grounds of food safety and business safety alike. As a matter of fact, this is what is so often practiced, intuitively. In this paper, this attitude is logically and systematically discussed, aimed at allowing its broad implementation.

The salient advantage of such a systematic rationale is the resulting documentation, which allows troubleshooting, in the short run, and skilful improvement cycles in the long run, as mandated by the ISO 9000 standards.

3.4. Prevention versus detection: A strategy enigma

Prevention is apparently better than detection, yet cost effectiveness considerations make this strategic enigma slightly more complex. Prevention of foreseen hazards is typically more effective in ensuring the safety and quality of the food products. It is of course much more expensive if investment is made in

hazard prevention, most of which will rarely materialize. Business-wise, nobody can afford to invest comprehensively in both the prevention and detection routes.

Practicing prevention alone, without detection, is highly risky in view of the fact that detection aims not only at foreseen hazards but also at some unexpected ones, which are difficult to cover by preplanned prevention measures. Hence, one should preferably seek an optimal balance between the prevention and detection routes. Even then, covering the entire spectrum of failure modes, many of which are redundant, is unrealistic for a viable food business. Hence, the salient question is how to prioritize the various hazards to be taken care of and how to decide which of them should engage prevention, which should preferably engage detection and which of them both prevention and detection. The following rationale may assist in formulating the optimal strategy:

(1) Raw materials (Table 1): Detailed protocols of harvesting, storage and transport can be highly cost effective in preventing most failure modes and hazards relevant to raw materials. Such protocols will minimize the number of detection control points necessary for the food product. Nevertheless, the applicability of such protocols is limited to well-established food products and processes. A newly developed product requires both extensive and even somewhat excessive prevention measures, as well as detection ones, aiming at discovering the actual hazards/pitfalls related to that particular product and concurrent processing, in particular the unexpected ones.

    Once the knowledge base of possible mishaps sufficiently increases, one may decrease the level of caution and the excessive portion of prevention and detection measures. In particular, one may decrease the detection measures related to foreseen hazards that do not materialize for a sufficiently long time under a wide variety of circumstances. Consequently, one may also consider, with utmost care, whether the preventive measures are just adequate, or constitute 'overkill'.

(2) Thermal processing (Table 1): Failure prevention in relation to thermal processing is significantly less expensive when compared with possible damage consequences. Hence, an extensive HACCP and the consequent control measures of temperature distribution and stability seem vital. In particular, measures should be taken to avoid the charring of food at the typically hot bottom of the cookware.

    A less straightforward branch of this failure prevention in thermal processing relates to the spoilage of frying oil owing to its repeated use. The long term hazard of carcinogen formation exceeds the short term one

of an off taste, yet both should be eliminated: both problems gravely increase following the radiation processing and the consequent chemistry. Routine sampling of the frying oil and off-line analysis seems onerous and costly.

Nevertheless, on-line optical gauging of the oil colour may offer a highly cost effective preventive measure. Once the process protocols are set to change the spoiled frying oil, or to recycle it, for example by a charcoal column or similar means, this problem may be cost effectively solved.

(3) Mechanical processing (Table 1): Failures in this respect are subtle changes in the food, and relate to undesired textural changes, leading to food chunks that substantially differ in size and/or texture from what is preferred by the customers. Prevention can be carried out by carefully monitoring the food texture, a non-trivial task. Intriguingly, monitoring the power consumption of the processing machines can give early warnings about overprocessing or underprocessing, as well as about loose metal parts that might have become detached from the machine!

(4) Packaging: Materials and processes related to packaging are often considered 'out of the scope' of the food per se. Nonetheless, packaging failure modes may result in scenarios far more serious than food failures, for two simple reasons: (i) almost no monitoring of the food is carried out once it is packaged and, (ii) handling of the packaged food is carried out with much less care than before packaging. The axiom that packaged food is no longer susceptible to contamination relays to an ideally fault free packaging, which does not exist. Hence, comprehensive packaging QA is a must when food is invlolved.

In particular, packaging integrity is important for irradiated food, for which an extended shelf life has been promised, and which cannot be compromised. In other words, the greater the promise, the greater the liability and QA requirements needed thereafter.

3.5. Why the special emphasis on radiation specific effects?

Radiation processing of food is nowadays well acknowledged as similar, in many aspects, to thermal processing (e.g. cooking, frying, baking) and does not imply any exceptional hazards. Nonetheless, radiation specific thinking is required for two major reasons:

(1) Specific knowledge of radiation chemistry in general, and radiation chemistry of foods in particular, is insufficiently disseminated. This situation necessitates that experts on radiation processing of food should

carry out the combined HACCP and thus explicitly analyse the radiation specific pitfalls, such as fat rancidization.

(2) Specific knowledge of the bacterial safety of food, irradiated long shelf life foods in particular, is also insufficiently disseminated.

Chemically preserved foods are only mildly sensitive to the opening of the packaging, as the preserving agent is still present. Radiation disinfection of foods leaves no preserving agent behind and, hence, their safety may be jeopardized if, after opening the packaging, the sanitary care is compromised. The unwary consumer, who is in the focus of the HACCP, must be made aware of this difference. This can only be achieved if the unwary producer is similarly made sufficiently aware of the above difference, via the HACCP and the radiation specific analysis therein. Thus, the producer can skilfully relay proper cautioning to its consumers, for their uncompromised safety, and for the producer's uncompromised business success.

3.6. A practical approach for optimizing HACCP implementation

The following eleven step methodology is suggested as a practical approach to attain an optimal HACCP for a food product aiming at a sustained business:

(1) Carry out a comprehensive HACCP analysis of the actual food to be prepared.
(2) Assess the most risky (less controlled) points in the process.
(3) Set the necessary QA system to minimize the need for control points.
(4) Set the necessary control measures, inspection points and inspection frequency.
(5) Run a few experimental preparations, employing markers for bacteria, foreign bodies and texture damage, etc., introduced at reasonable points.
(6) Analyse the effectiveness of the HACCP system.
(7) Improve the HACCP set, add missing control points and remove redundancy.
(8) Run validation preparations as before.
(9) Write the protocol and manual for performing the HACCP.
(10) Train the HACCP personnel, verify their knowledge and certify accordingly.
(11) Start production.

4. CONCLUSIONS

The combined multi-risk HACCP assessment seems capable of providing a broad and comprehensive picture of the potential failure modes and their consequent risks to food. Special emphasis on assessing the risks for radiation pasteurized foods provided the key control points for minimizing these hazards, as well as promoting adequate awareness of producers and consumers, to the specifics of handling such processed foods.

A cost effectiveness driven critical review of the preventive versus detection measures is crucial to obtain a cost effective safe product and thus a sustained business. The pragmatic approach should back the theoretical assessment with experimental controlled production, to verify which parts of the HACCP are necessary, which are missing, and which are redundant. Finally, the HACCP, in common with the food production it accompanies, should advance with time and experience gained, to provide both increased safety and reduced safety costs.

PART II: UTILIZATION OF THE METHODOLOGY FOR IRRADIATION OF ETHNIC READY-TO-EAT MEALS

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Abstract

A multiparameter hazard analysis and critical control point methodology for foods under development has been described in Part I. It focused on a systematic fourfold assessment of safety hazards, sensorial failures, economic adversities and radiation specific pitfalls, and the critical control points for their early detection and/or mitigation. It attempted also at combining these four parameters to form a qualitative prioritization of the numerous control points, to screen the most cost effective ones for implementation. The further development of the methodology described in this report demonstrates its application for ethnic foods. These foods are often characterized with multi-ingredient recipes and multiple preparation steps, subtle ensemble of specific flavours, as well as relatively high bacterial loads. A group treatment of these ingredients has been demonstrated on several ready-to-eat foods investigated by Indonesian researchers. This approach has been found greatly effective in pinpointing the primary control points of the prepared ethnic meals. The primary achievement resulting from this approach is the insight that a two stage irradiation is apparently the best solution to attain both bacteria eradication and taste/flavour conservation or, in other words, the desired combination of high levels of safety and high quality.

1. INTRODUCTION

The methodology of hazard analysis and critical control points (HACCP) is employed in the food industry almost solely for foods under production, typically following a regulatory demand. It is rarely carried out for foods under R&D, since foods are still being considered 'simple products' despite their ever increasing complexity and innovation.

In contrast, in high-tech products, space systems in particular, the contemporary practice is to start the risk assessment as soon as the first design is complete, and develop the risk assessment hand in hand with the products' evolution. The rationale behind this practice is to eliminate higher risk designs as soon as possible, before undue time, effort and finance are invested. Food

products in general, and prepared meals in particular, are nowadays moving ever closer to high-tech ones. Hence, the rationale of HACCP at the R&D stage of innovative foods seems more viable in general, and for long shelf life foods in particular. The adaptation of the HACCP from a single parameter methodology, aimed at production only, into to a four parameter one, aimed at R&D as well, has been demonstrated in Part A. In Part B, several ethnic radiation pasteurized prepared meals, which have been studied in Indonesia, were analysed by the modified HACCP.

The prepared meals of Indonesia, as well as numerous other ethnic foods, are characterized with a very long list of ingredients and preparative processes. This list seems too long for utilizing the detailed ingredient by ingredient assessment that has been done for more simple foods. Hence, a further modified HACCP methodology, adapted for multi-ingredient prepared meals, was apparently needed. The modification employed is demonstrated and discussed, and the conclusions and benefits derived thereby are assessed.

2. METHODOLOGY

2.1. Ingredients grouping

Table 1, copied from Ref. [1], shows the numerous ingredients used in Indonesian ethnic soups. For these types of food, a group by group assessment methodology seemed crucial and, hence, was developed. The primary assessment groups are: animal source ingredients, of which the major hazard is bacterial and health compromising; fat-rich ingredients, including frying oils, the primary hazard of which is rancidization and sensorial degradation; anti-bacterial ingredients/spices, the primary concern related to these being uncontrolled concentration of active materials, and uncontrolled bacterial and sensorial quality. All other groups are of minor related hazards.

2.2. Combined HACCP of ingredient groups

The assessment of the various hazard elements was carried out by employing the same methodology presented in Part I (see Table 1 in Part I). Only the primary ones which justify inspection and/or preventive actions have been listed. The assessment is based on the assumption that the prepared meals are being produced, whether by a food laboratory or a food industry, while appropriately conforming with the fundamental prerequisites of good hygiene and manufacturing practices, as well as good irradiation practice.

TABLE 1. INGREDIENTS FOR PREPARING INDONESIAN ETHNIC SOUPS ON A LABORATORY SCALE

Ingredients	Soup (quantity)			
	Black 20 × 300 g	Oxtail 20 × 300 g	Chicken vegetable 18 × 200 g	Chicken sweetcorn 25 × 250 g
Beef	2.5 kg			
Oxtail		2 kg		
Chicken			3 kg	3 kg
Football fruit (*Pangium edule)*	25 nuts			
Shallot	175 g	70 g	105 g	
Garlic	35 g	35 g	30 g	
Roasted coriander	5 teaspoons			
Roasted candlenut	25 pieces			
Seedless red chilli	5 pieces			
Ginger	10 cm			
Small aromatic lemon leaf	10 pieces			
Roasted fish paste	3 teaspoons			
Turmeric	10 cm			
Ginger root	10 cm			
Lemon grass	5 pieces			
Spice *(Kaempferia galanga)*	10 cm			
Salt	4 teaspoons	5 teaspoons	5 teaspoons	5 spoons
Palm sugar	3 teaspoons			
Bay leaf	10 pieces			
Palm oil	1 L	500 mL		
Water	5 L	5 L	3 L	3 L
Onion		5 pieces		
Ground nutmeg		4 teaspoons	2.5 teaspoons	5 teaspoons
Cloves		20 pieces		
Ground white pepper		5 teaspoons	4 teaspoons	
Onion leaf		5 pieces	10 pieces	

TABLE 1. INGREDIENTS FOR PREPARING INDONESIAN ETHNIC SOUPS ON A LABORATORY SCALE (cont.)

Ingredients	Soup (quantity)			
	Black 20 × 300 g	Oxtail 20 × 300 g	Chicken vegetable 18 × 200 g	Chicken sweetcorn 25 × 250 g
Celery		5 pieces	10 pieces	
Margarine		2 spoons	5 spoons	
Sugar (cane)			4 teaspoons	
Young sweetcorn				20 pieces
Chicken sausage				1.1 kg
Carrot			450 g	350 g
Eggs				7
Corn starch				10 spoons
Green beans			350 g	
Broccoli			400 g	
Frozen sugar peas			500 g	

3. RESULTS AND DISCUSSION

Table 2 lists the primary ingredients of the group carrying the highest bioburden, namely, ingredients from animal sources. Table 3 lists the primary HACCP elements of these ingredients which, importantly, carry typical bacterial loads of 10^7 CFU/g and over [1].

The foremost hazard, demonstrated in Table 3, is the high bacterial load in the animal source ingredients, which poses a grave cross-contamination risk to the manufacturing line. This assessment suggests that a pre-pasteurization stage, used separately for animal source ingredients, may be advantageous. This preventive measure can be achieved by the manufacturer of the prepared meals implementing a two stage irradiation. In other words, pre-pasteurization of all highly contaminated ingredients may be greatly beneficial in reducing the cross-contamination risk. Practically, the ingredients carrying high bacterial loads can be pasteurized by radiation as part of the production strategy employed by their suppliers, e.g. packed frozen meats can be irradiated prior to supplying them to the prepared meals factory.

Such a strategy may allow a much lower dose to be used for the pasteurization of the whole prepared meal, at the last stage, and, possibly,

TABLE 2. ANIMAL SOURCE INGREDIENTS FOR PREPARING INDONESIAN ETHNIC SOUPS

Ingredients	Soup (quantity)			
	Black 20 × 300 g	Oxtail 20 × 300 g	Chicken vegetable 18 × 200 g	Chicken sweetcorn 25 × 250 g
Beef	2.5 kg			
Oxtail		2 kg		
Chicken			3 kg	3 kg
Chicken sausage				1.1 kg
Roasted fish paste	3 teaspoons			
Eggs				7

TABLE 3. COMBINED HACCP FOR ANIMAL SOURCE SOUP INGREDIENTS

Raw materials: Animal source							
Control point	Failure mode/ hazard	Preventive measures	Detectability	Haz.	Sens.	Econ.	Rad. spec.
Bacteria	Very high loads	Microbial test Two stage irradiation	OK	✓✓			
Fat	Fat loads and rancidization upon irradiation	Fat removal	OK		✓✓		✓✓
Bones	Bone fragments: Internal wounds and bleeding	Bone removal	Only of large fragments!	✓✓	✓✓	✓✓	

better preservation of the sensorial quality of the food. Nonetheless, any initiative of two stage irradiation must be accompanied by adequate research, to assess the possible formation of undesirable compounds. Further, a proper regulation allowing two stage irradiation must be implemented, since all the currently existing regulations still abide by the now obsolete concept that re-irradiation is harmful and hence has been banned. The 'high dose' committee [2] has established an overwhelming rationale that the sensorial quality of irradiated foods will degrade at irradiation doses orders of magnitude lower

than those which may pose any radiation related health risk. Hence, once non-compromising bacterial safety and sensorial quality are targeted, the radiation dose and the number of irradiation stages are no longer issues of any specific concern.

Another hazard shown in Table 3, the bone residuals, is non-radiation specific, but rather soup specific: people tend to swallow the soup 'as is' and therefore bone residuals pose a much higher risk than in solid meals that require chewing and, hence, provide the consumer sufficient time and sensorial experience to detect bone fragments before swallowing the food.

The fat hazard mentioned in Table 3 in relation to animal fat and rancidization is further addressed in Tables 4 and 5, which relate to all non-animal source, fat-rich ingredients of the prepared meals.

The primary hazard related to the fat content in the food is the sensorial one, rather than immediate health risk. Nonetheless, of particular economic impact (Table 5) is the radiation induced long term development of rancidization products (last row in Table 5). Intermediate species formed during the irradiation gradually develop into oxidized products with the typical 'off' taste of rancidization. The grave economic risk related to this long term sensorial failure stems from the fact that the 'price tag' for this failure is the highest: the entire product costs, packaging and shipment costs, inventory costs and loss of reputation, since this type of failure is a consumer detected one.

It is noteworthy that proper oxygen free packaging of the food can help minimize the rancidization processes. Nevertheless, minimizing the radiation dose for the fat-rich ingredients can also help in minimizing the rancidization, well in accord with the two stage irradiation rationale.

TABLE 4. FAT-RICH INGREDIENTS FOR PREPARING INDONESIAN ETHNIC SOUPS

Ingredients	Soup (quantity)			
	Black	Oxtail	Chicken vegetable	Chicken sweetcorn
	20 × 300 g	20 × 300 g	18 × 200 g	25 × 250 g
Roasted coriander	5 teaspoons			
Roasted candlenut	25 pieces			
Margarine		2 spoons	5 spoons	
Palm oil	1 L	500 mL		

TABLE 5. COMBINED HACCP FOR FAT-RICH INGREDIENTS

Raw materials: Fats and oils							
Control point	Failure mode/ hazard	Preventive measures	Detectability	Haz.	Sens.	Econ.	Rad. spec.
Degraded fat	Oil oxidation of fried ingredients	Temperature and 'ecycling' control	Mainly sensorial	✓	✓✓		
Degraded fat	Oil charring of fried ingredients	Temperature and recycling control	Mainly sensorial	✓	✓✓		
Degraded fat	Radiation induced rancidization	Minimize quantity of fat and frying fat	Mainly sensorial	✓	✓✓	✓✓	✓✓

The rancidization effected hazard seems economically alarming in view of its manifestation in the final product at the end of storage, whereas the capability to predict it is currently poor. Running the updated and modified HACCP on the various ethnic foods investigated in this coordinated research project demonstrated the complexity of this issue, and the insufficient knowledge of the interplay of radiation, oxygen and antioxidants on the final level of rancidization and the resulting off flavours. Further importance of the rancidization factor stems from the fact that fat rich parts of the meat are typical of the diet of impoverished people. Hence, fat is typical in many ethnic foods around the world and addressing rancidization properly should be a high priority.

HACCP related to ingredients with potential antibacterial activity is displayed in Tables 6 and 7. While these ingredients per se may impede the growth of bacteria, during irradiation some of them can also impede the actual killing of the bacteria by the radiation and, hence, necessitate higher doses to obtain the desired reduction in bacterial loads.

The principal objective is to lower the bacterial loads in the prepared meals below a certain limit, at a constant dose, and to maintain a predictable constant shelf life of the prepared meals thereafter. However, the seasonal variance of active materials in the above ingredients may hamper this stability, which depends on consumer acceptance. Hence, attempts should be made to compensate for these seasonal variances in antibacterial activity and consequently flavour. Detecting concentrations of the active materials in the raw materials is essential for that purpose, and compensation by quantity changes, as needed, can solve the problem.

TABLE 6. INGREDIENTS WITH ANTIBACTERIAL ACTIVITY IN INDONESIAN ETHNIC SOUPS

Ingredients	Soup (quantity)			
	Black 20 × 300g	Oxtail 20 × 300g	Chicken vegetable 18 × 200g	Chicken sweetcorn 25 × 250g
Seedless red chilli	5 pieces			
Garlic	35 g	35 g	30 g	
Ginger root	10 cm			
Ground white pepper		5 teaspoons	4 teaspoons	

TABLE 7. COMBINED HACCP FOR INGREDIENTS WITH POSSIBLE ANTIBACTERIAL ACTIVITY

Raw materials: Ingredients with possible antibacterial activity							
Control point	Failure mode/ hazard	Preventive measures	Detectability	Haz.	Sens.	Econ.	Rad. spec.
Antibacterial agent	Seasonal variations in concentration	Test concentration of active agent compensate	?	✓✓		✓✓	✓✓
Antibacterial agent	Radiation destruction	Test concentration of active agent compensate	?	✓✓		✓✓	✓✓

The possible interference of these ingredients in bacteria eradication is a serious drawback. It suggests, once again, that the major bacteria eradication exercise should take place before adding ingredients which may protect the bacteria, another rationale supporting the strategy of two stage irradiation. This complexity is further demonstrated in Tables 8 and 9, which relate to a large number and variety of herbs and flavouring vegetables.

The salient features of the list of ingredients in Table 8 are their numbers and variance. Their ripening takes place at different seasons and, hence, fresh products will exhibit great variance in taste from one season to another. This

TABLE 8. VEGETABLES AND HERBS USED IN INDONESIAN ETHNIC SOUPS

Ingredients	Soup (quantity)			
	Black 20 × 300g	Oxtail 20 × 300g	Chicken vegetable 18 × 200g	Chicken sweetcorn 25 × 250g
Football fruit (*P. edule)*	25 nuts			
Shallot	175 g	70 g	105 g	
Lemon leaf	10 pieces			
Lemon grass	5 pieces			
K. galanga and turmeric	10 cm each			
Bay leaf	10 pieces			
Onion		5 pieces		
Cloves		20 pieces		
Onion leaf		5 pieces	10 pieces	
Celery		5 pieces	10 pieces	
Young sweetcorn				20 pieces
Carrot			450 g	350 g
Green beans			350 g	
Broccoli			400 g	
Frozen sugar peas			500 g	

TABLE 9. COMBINED HACCP FOR VEGETABLES AND HERBS

Raw materials: Vegetables and herbs							
Control point	Failure mode/ hazard	Preventive measures	Detectability	Haz.	Sens.	Econ.	Rad. spec.
Microbial	Contamination by birds, rodents insects, etc.	Microbial testing of many different ingredients	Microbial and aflatoxin testing of the product	✓✓	✓✓	✓✓	✓✓
Physical	Foreign matter, e.g. stones, metal, shells	Careful inspection and sieving	Difficult at the industrial scale	✓✓	✓✓	✓✓	

variance may hamper the sensorial stability which is important for consumer acceptance, as well as the radiation effected changes. Of course, this variance can be minimized by employing frozen ingredients, albeit at a severe increase in costs. These factors require thorough consideration by the respective industry when planning their raw materials supply chain.

The large variety of vegetables and herbs, typical of ethnic foods, calls for special care regarding the irradiation pasteurization. Ethnic foods are often characterized by a very rich flavour, catering to the taste of the local consumers. Hence, sensorial damage by overirradiation is a distinct risk and, hence, it is essential to try and lower the dose applied to these flavour producing ingredients. In view of the relatively large dose necessary for attaining the acceptable safety level of other ingredients, the two stage irradiation seems advantageous.

As for the HACCP for each and every ingredient per se, it would be quite a tedious task to monitor all of these, even on a laboratory scale, let alone an industrial one. Nonetheless, nowadays, the supply chain of herbs and vegetables is becoming more and more industrial. Many of the ingredients in Table 8 can nowadays be ordered and purchased cut, cleaned, inspected and packed under modified atmosphere, as well as under cooling conditions. If purchased from a certified industrial producer of these intermediate products, the manufacturer of the prepared meals can save much effort, expense and risk.

In the specific case of soups, water is a major constituent by weight, with a potentially strong effect on both the bacterial load and the sensorial quality. Ordinary tap water in some places may not be sufficiently wholesome from bacterial, sediment and off taste considerations, to use it for preparing high quality and safe prepared meals. Nevertheless, on an industrial scale, a water treatment facility is an acceptable requirement, rather a common one, in this industry. Hence, the water quality issue on the HACCP list can be answered with standard good manufacturing practice methods.

Likewise, most of the ingredients in Tables 10 and 11 can nowadays be ordered and purchased from a certified industrial source and, hence, no longer be a cause for concern. However, Table 12, which relates to all the ingredients, draws attention to a fundamental difference between laboratory scale rationale and an industrial scale one.

The key factor, often overlooked at the food laboratory scale of ‘production’, is product reproducibility. Once consumer trust in a new product has been gained, typically owing to sensorial attributes, changes to the product are risky. Changes to a product may result in consumers abandoning it, since it may no longer meet their sensorial expectations and preferences. That is why it is essential, well before attempting to transfer the new prepared meals to industrial production, to ‘seal’ all the quantity loopholes that remain in the food recipe.

TABLE 10. POWDERED INGREDIENTS AND LIQUIDS IN INDONESIAN ETHNIC SOUPS

Ingredients	Soup (quantity)			
	Black 20 × 300 g	Oxtail 20 × 300 g	Chicken vegetable 18 × 200 g	Chicken sweetcorn 25 × 250 g
Corn starch				10 spoons
Sugar (palm)	3 teaspoons			
Sugar (cane)			4 teaspoons	
Salt	4 teaspoons	5 teaspoons	5 teaspoons	5 spoons
Water	5 L	5 L	3 L	3 L

TABLE 11. COMBINED HACCP FOR POWDERED INGREDIENTS AND LIQUIDS

Raw materials: Powders and liquids							
Control point	Failure mode/ hazard	Preventive measures	Detectability	Haz.	Sens.	Econ.	Rad. spec.
Microbial	Contamination following inadequate storage	Microbial testing	OK	✓	✓	✓	
Physical	Foreign matter	Careful inspection and sieving	Difficult at an industrial scale	✓✓	✓✓	✓✓	

TABLE 12. ALL INGREDIENTS IN INDONESIAN ETHNIC SOUPS

Ingredients	Soup (quantity)			
	Black	Oxtail	Chicken vegetable	Chicken sweetcorn
All	XX pieces	YY teaspoons	ZZ cm leaf	

TABLE 13. COMBINED HACCP FOR ALL INGREDIENTS

Raw materials: Fats							
Control point	Failure mode/hazard	Preventive measures	Detectability	Haz.	Sens.	Econ.	Rad. spec.
XX pieces, YY cm	Undefined weight can drastically alter the taste, the bacterial load or the antibacterial activity	Define weight acceptable limits, e.g. 15–18 g	OK	✓	✓✓	✓✓	✓✓

For radiation pasteurized foods, this aspect is of greater importance, in view of the recently established consumer acceptance. It is essential to remain alert to the risk that taste changes may be attributed to public perception of the 'bad effects of radiation' and result in rumour, panic and long lasting damage to consumers' acceptance. In other words, product reproducibility and stability of sensorial quality are 'musts' in relation to all so-called high-tech foods, irradiated ones in particular.

As with the need to practice accurate quantity control in the food preparation so there is a need for accurate process control, as demonstrated in Tables 14 and 15. Without the latter, the stability of the sensorial quality is at stake. It should be emphasized again that this industry related consideration needs to be addressed at the R&D stage, rather than at a later stage.

Last, but not least, comes the prepared meal packaging and its related hazards. If the food in question involves multistage industrial processing, intermediate packaging of the semi-processed ingredients and the final packaging of the entire prepared meal should be carried out. The intermediate packaging needs to eliminate contamination between process stages, as well as spoilage due to loss of flavour, gradual oxidation, or changes in moisture levels.

The typical shelf life desired for intermediate packaging is a few days unless an intermediate semi-processed ingredient is, by itself, a product which is being transported from one industry to another. On the other hand, the final packaging is required to allow a rather prolonged shelf life, under sales and consumer handling, without compromising safety and quality.

3.1. The two stage irradiation strategy: Pros and cons

The potential strategy of two stage irradiation discussed so far is rather new and, hence, insufficiently tested and assessed. In view of its great promise, it is timely to discuss its possible drawbacks, as well as viable alternatives, as

TABLE 14. ALL INGREDIENTS IN INDONESIAN ETHNIC SOUPS

Ingredients	Soup			
	Black	Oxtail	Chicken vegetable soup	Chicken sweetcorn
All	Frying, cooking	Cutting	Rinsing	

TABLE 15. COMBINED HACCP FOR ALL PREPARATION PROCESSES

All ingredients							
Control point	Failure mode/ hazard	Preventive measures	Detectability	Haz.	Sens.	Econ.	Rad. spec.
Temperature, time, covering	Loss of volatile ingredients	Needing to be defined: Acceptable limits of cooking/frying temperature and duration Cover technique (or pressure cooking) Rinsing protocols (must be well defined)	OK	✓✓	✓✓	✓✓	✓✓

TABLE 16. PACKAGING OF INGREDIENTS AND PREPARED MEALS OF INDONESIAN ETHNIC SOUPS

Ingredients	Soup			
	Black	Oxtail	Chicken vegetable	Chicken sweetcorn
Intermediate preparations, final prepared meal	Packaging	Packaging	Packaging	Packaging

TABLE 17. COMBINED HACCP FOR PACKAGING

Raw materials: Fats							
Control point	Failure mode/ hazard	Preventive Measures	Detectability	Haz.	Sens.	Econ.	Rad. spec.
Packaging QA	Packaging failure: Microbial contamination	QA 1.2 QC Microbial testing	OK	✓✓	✓✓	✓✓	✓✓
	Oxygen penetration	Obtain COC and COT from the packaging producer on oxygen permeability	OK		✓✓		✓✓
	Humidity loss	As above regarding water vapour permeability	OK		✓✓		

shown in Table 10. Essentially, the principle of two stage irradiation is that raw materials which are bacterially contaminated beyond acceptable levels will be irradiated (under optimal conditions) before being admitted into the food factory. This is particularly important for animal source raw materials.

Nevertheless, the currently available ensemble of bacterial eradication technologies, besides ionizing irradiation, includes heating, hydrostatic pressure and other alternatives. Hence, irradiation should preferably be considered as part of the ensemble of bacteria eradication technologies and not as the sole method, in order to prevent its misuse, overuse and abuse. Skilful optimized orchestration of the various methods, at the right dose and sequence, should be tailored for each raw material, intermediate preparation or prepared meal. Such a scientifically based attitude should aim at the highest levels in terms of safety, shelf life and quality, as well as at the most cost effective route. A skilfully operated HACCP, one that accompanies the various alternatives investigated, already at the R&D stages, can assist in decision making and result in a cost effective R&D, and then yield the most cost effective product and production route specifications.

Approaching the industrial production stage of safe prepared meals was further emphasized during this study of applying the modified HACCP to ethnic foods. In view of the currently accelerating consumer demand and

TABLE 18. PROS, CONS AND ALTERNATIVES OF THE TWO STAGE IRRADIATION OF PREPARED MEALS

Issue of concern	Pros	Cons	Alternatives
Cross-contamination from contaminated raw materials	Cross-contamination risks are reduced, prevention and detection costs are reduced	Additional costs of packaging, transport and logistics	Cross-contamination focused HACCP, prevention and detection
Increased final dose due to protection of bacteria by anti-oxidants	Overall lower doses to achieve safe levels of bacteria	Possible bacteria recovery between irradiation stages	Combination of heat, pressure and irradiation to eradicate bacteria
Increased off flavours, e.g. rancidization	Overall lower doses minimize rancidization	Possible rancidization between irradiation stages, and strong off flavour thereafter	Active charcoal canisters to trap volatile off flavour molecules
Texture spoilage Speciality flavour loss	Overall lower doses minimize texture and flavour damage	Elapsed time between irradiation stages and extra handling may increase texture damage	Use of additives for texture toughening and flavour enhancement
Costs	Lower weight × dose costs	Higher logistical costs	Contract stipulation of clean raw materials from the suppliers

industrial production of prepared meals and convenience foods, the multistage treatment approach to ensure ingredient safety seems favoured on the basis of safety, taste and smell, irradiation (or alternative methods) and economics. Optimizing bacteria eradication along the production stages can achieve better food quality and safety, while practically using less food treatment, but at two or more sequential stages.

4. CONCLUSIONS

The combined multirisk HACCP assessment, carried out through the R&D of novel foods, is suitable not only for pinpointing the most critical

control points, but also for identifying potentially advantageous production pathways.

In view of the relative complexity and variety of ingredients in ethnic foods, the combined HACCP is effective in pinpointing their specific critical control areas: (i) animal source ingredients (bacterial loads), (ii) fat rich ingredients (rancidization potential), (iii) antibacterial active ingredients (seasonal variations and microbial load impact) and (iv) flavour rich ingredients (radiation sensitivity and sensorial quality impact).

Two stage irradiation of ethnic foods, at the individual ingredient stage and the final stage, was found to be a highly valuable approach. It deserves further study and regulatory steps, as well as skilful orchestration with non-irradiative pasteurization methods, in order to attain the optimal food products and their processing sequences.

Food R&D HACCP seems a novel way to direct food R&D progress effectively. Nonetheless, the industrial scale considerations and rationale should already be implemented, as well as the economic ones, customer acceptance in particular. The HACCP should thus encompass all the facets of food R&D to ensure its safety, quality and marketability, particularly for complex and innovative foods such as prepared ethnic meals.

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MICROBIAL INACTIVATION AND SHELF LIFE EXTENSION OF KOREAN TRADITIONAL PREPARED MEALS BY IRRADIATION

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Abstract

The effects of gamma irradiation on microbial inactivation and shelf life extension of Korean traditional prepared meals, including bulgogi and its sauce, marinated beef rib and Kimbab, were investigated. Raw vegetables, fruits and soy sauce used for making bulgogi sauce were highly contaminated and most of them with *Bacillus* spp. and coliform bacteria at the initial stage. Irradiation at 10 kGy eliminated coliforms in the bulgogi sauce and no growth was found during the 4 weeks of storage at 20°C. The sensory evaluation of bulgogi and its sauce showed that the colour of irradiated samples was better than that of non-irradiated controls or heat treated samples. The total bacteria count and coliform of marinated beef ribs were 5.68 and 3.68 ln CFU/g, respectively. The effect of irradiation on the growth of the four test microorganisms inoculated (about 10^6–10^7 CFU/g) into the marinated beef ribs were dose dependent (a higher dose produced a greater reduction). All the four pathogens inoculated on marinated beef ribs were eliminated at 4 kGy. The D_{10} values of *Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium* and *Escherichia coli* on inoculated marinated beef ribs were 0.66 ± 0.01, 0.59 ± 0.05, 0.64 ± 0.02 and 0.54 ± 0.01 kGy, respectively. Of these, *E. coli* was the most radiation sensitive in the raw marinated beef ribs. The number of the four pathogens inoculated into Kimbab decreased by 2–3 ln CFU/g for every 1 kGy increment and were not detected after 3 kGy. The D_{10} values of pathogens inoculated into the Kimbab were in the range 0.31–0.44 kGy. This study indicated that irradiation is effective in ensuring the safety of Korean traditional prepared meals, including bulgogi sauce, bulgogi, marinated beef ribs and Kimbab with acceptable sensory quality. For Kimchijumeokbab, viable cells of the four pathogens inoculated increased slightly after abusive storage condition (20°C), although under commercial condition (4°C), and after 2 weeks of storage, no viable cells were detected. A 3 kGy dose was the threshold dose for this meal from the sensorial viewpoint. This dose must be applied with cold storage in order to improve the safety and ensure a shelf life extention of Kimchijumeokbab.

1. INTRODUCTION

Owing to the expansion of the convenience food industry, mainly by the development of the catering services and distribution system, the importance of the hygienic quality of prepared food during the preparation, processing, handling and storage has increased. Nowadays, prepared foods are widely available on the market all around the world, but at the same time, they have been the cause of major outbreaks of foodborne diseases. In the United States of America, overall incidences of pathogens in cooked ready-to-eat meat products were 8.1% over the period 1993–1996 [1]. A survey of ready-to-eat cooked meat products in Germany showed an incidence rate of 3.7% for *Listeria monocytogenes* [2]. Mytle et al. [3] reported that illnesses have resulted when supposedly ready-to-eat foods were not reheated before consumption. It has also been reported that raw beef, soy sauce and raw vegetables may harbour potential foodborne pathogens [3–5]

In the Republic of Korea, *Salmonella* spp. in Kimbab was detected at 623 CFU/g on an average of about 214 samples and the frequency of detection in the summer was higher than in the winter season [6].

The World Health Organization [7] reported that gamma irradiation technology has positive effects in preventing decay thereby killing microorganisms and by improving the safety and shelf stability of food products. The technology has been officially adopted for its wholesomeness and economic benefits.

The objective of the present study was to demonstrate the efficacy of irradiation treatment in eliminating the potential pathogens of public health significance in typical Korean prepared meals, such as bulgogi and its sauce, marinated beef ribs, Kimbab and Kimchijumeokbab, the popular types of prepared product in the Republic of Korea, thereby ensuring the safety and extending the shelf life of the products.

2. MATERIALS AND METHODS

2.1. Preparation of sample

Samples of bulgogi sauce, bulgogi, marinated beef ribs and Kimbab were prepared immediately before the experiment. Freshly prepared marinated beef ribs (deboned, uncooked Galbi), Kimbab (steamed rice rolled in dried laver) raw materials for bulgogi sauce (soy sauce, garlic, onion, green onion, sugar, kiwi, sesame oil, black pepper and grain wine) and meat for making bulgogi were purchased from a local store.

A portion of the prepared bulgogi sauce was heated at 100°C for 30 min and another portion was packaged separately in a polyethylene bag ready for irradiation. A non-irradiated and non-heat-treated sample was also prepared as a control. The sauces that were treated by heat or irradiation (2.5, 5.0 and 10 kGy) were analysed for microbiological quality, pH and protease activity. Sliced meat was marinated with each prepared sauce in a 2:1 ratio for 3 h in a refrigerator (4°C) and cooked for sensory analysis.

The marinated beef ribs and Kimbab were sliced (1 cm thickness) aseptically and packaged (10 g) in oxygen impermeable nylon bags (2 mL $O_2 \cdot m^{-2} \cdot 24\ h^{-1}$ at 0°C, 0.09 mm thickness (Sunkyung Co. Ltd., Seoul). The samples were exposed to an irradiation dose of 30 kGy (AECL, IR-79, MDS Nordion) at 12 ± 0.5°C to achieve the complete inactivation of the indigenous microflora.

For artificial contamination, Kimchijumeokbab was prepared with steamed rice, Kimchi, sugar, sesame oil, hot pepper powder and fried pork, which was prepared with grain wine, green onion, garlic, sesame seed, ginger, pepper, soy sauce, sugar and sesame oil.

2.2. Strains and culture condition

For the inoculation test, *Staphylococcus aureus* (KCTC 1916), *Bacillus cereus* (KCTC 1012), *Salmonella typhimurium* (KCTC 1925), *Escherichia coli* (KCTC 1682) and *Listeria ivanovii* were obtained from a Korean collection for type culture (KCTC, Daejeon). Of these, *E. coli, S. aureus* and *L. ivanovii* were grown in a tryptic soy broth (Difco Laboratories, Sparks, USA), and *S. typhimurium* in a nutrient broth (Difco Laboratories). The incubation temperature for *E. coli, S. aureus* and *S. typhimurium* was 37°C, and for *L. ivanovii* it was 30°C. The bacterial cultures were prepared for 24 h in a sterilized broth medium from one colony from an agar slant, from which 0.1 mL was transferred to a new broth medium and grown for 18 h. The cultures were centrifuged (2795g for 10 min at 4°C) in a refrigerated centrifuge (Vs-5500, Vision Scientific Co., Seoul). Cultures were washed twice with sterile peptone water. The pellet was finally suspended in sterile peptone water to achieve a cell density above the 10^7~10^8 CFU/g levels.

2.3. Inoculation of samples with test organism

Packaged marinated beef ribs and Kimbab were inoculated with 10^6–10^7 CFU/g of the four pathogens and later stored. The test culture suspension (200 μL) was uniformly and aseptically spread and kept in a sterile workstation for 10 min to allow it to be absorbed. The samples were then packed into sterile stomacher bags and sealed.

2.4. Irradiation

Packed marinated beef ribs and Kimbab inoculated with the test organisms, bulgogi sauce and cooked bulgogi were treated in a ^{60}Co irradiator (AECL, IR-79, MDS Nordion) at the Korea Atomic Energy Research Institute in Daejeon. An ice bag (1 cm thickness) covered the sample holder to avoid a temperature increase of the sample during irradiation. The source strength was approximately 100 kCi with a dose rate of 10 kGy/h at 12 ± 0.5°C. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany) and the free radical signal was measured using a Bruker EMS 104 EPR analyser. The dosimeters were calibrated against an international standard set by the IAEA. The doses applied in this study were 1, 2, 2.5, 3, 4, 5 and 10 kGy for different prepared foods. After irradiation, the marinated beef ribs were stored under two different conditions: (i) commercial refrigeration (4°C) and (ii) abusive temperature (20°C). The Kimbab samples were stored at 10, 20 and 30°C for 24 h post-irradiation.

2.5. Microbiological analysis

The samples (10 g each) were aseptically homogenized for 2 min in a sterile stomacher bag containing 90 mL of sterile peptone water using a stomacher laboratory blender (Model 400, Tekmar Co., Cincinnati, USA). The media used for enumeration of the coliform bacteria, *Salmonella–Shigella* spp., *S. aureus*, *Bacillus* spp., *B. cereus*, *S. typhimurium* and *E. coli* were prepared by dextrose tryptone agar (Difco), SS agar, Baird Parker agar (Difco), cereus selective agar (Oxoid, Basingstoke, United Kingdom), xylose lysine deoxycholate agar (Difco) and eosin methylene blue agar (Difco), respectively. The incubation temperatures for *Bacillus* spp., *L. ivanovii* and the others were 50, 30 and 37°C, respectively, for 48–72 h. The colony forming units per gram were counted and the D_{10} values determined. Experiments with each bacteria culture were conducted independently, twice.

2.6. pH and relative neutral protease activity

The pH was measured using a pH meter (Model 520A, Orion Research Inc., Boston, USA) by adding 9 parts of deionized distilled water (DDW) to 1 part of sample homogenate. Relative neutral protease activity was measured by the method developed by Kim et al. [8] and modified. A 10 g portion of the bulgogi sauce was homogenized with 90 mL of DDW using a blender (Hanil, FM680T, Seoul) for 60 s and left to stand for 30 min at 4°C, and then filtered with filter paper (No. 3, Whatman International, UK). The filtrate was used for

the protease activity. The substrate used was casein (0.5%, dissolved in 50mM phosphate buffer, pH6.0) and the released tyrosine was determined. One unit of enzyme activity was defined as the amount of enzyme required to liberate 1 μmol tyrosine/min.

2.7. Sensory analysis

A consumer test (n = 33) was used to evaluate the sensory quality of the bulgogi sauce and the cooked bulgogi. For the sauce, the odour and the colour were measured using a dish holding 50 mL of sauce. The cooked bulgogi was marinated with the sauce in a 2:1 ratio and the marinated beef was placed in a preheated pan at about 170°C and cooked for about 8.5 min to reach a temperature of approximately 78°C. After cooling for 2 min at room temperature, a 20 g sample was served to the panellists individually. A 9 point hedonic scale was provided for the panellists to assess as follows: like extremely (9), like very much (8), like moderately (7), like slightly (6), neither like nor dislike (5), dislike slightly (4), dislike moderately (3), dislike very much (2), and dislike extremely (1). The sensory parameters for the cooked bulgogi were colour, odour, taste and tenderness.

2.8. Statistical analysis

Each data represents the mean of two different experiments with three measurements in each experiment. Mean values and standard deviations were calculated using a statistical analysis system [9] and recorded.

3. RESULTS AND DISCUSSION

3.1. Bulgogi and bulgogi sauce

Raw vegetables and fruits used for making bulgogi sauce exhibited high levels of bacterial contamination (Table 1). *Bacillus* spp. was detected in all ingredients except for grain wine, in a range of 10^2–10^5 CFU/g, and coliform and *Salmonella–Shigella* were also found in the garlic and onion samples. These results indicated that the contamination of the raw materials was affecting the hygienic quality and the safety of the prepared bulgogi sauce.

The growth of *Bacillus* spp. in bulgogi sauce during one month of storage at 20°C is shown in Fig. 1.

The control, which was not irradiated or heat treated, and heat treated bulgogi sauce showed a rapid growth of *Bacillus* spp. No experiments were

TABLE 1. MICROBIOLOGICAL CONTAMINATION OF RAW MATERIALS USED IN THE PREPARATION OF BULGOGI SAUCE[a]

Raw materials	Microbes (CFU/g)		
	Bacillus spp.	Coliform	*Salmonella–Shigella* spp.
Soy sauce	3.77×10^3	n.g.[b]	n.g.
Grain wine	n.g.	n.g.	n.g.
Sugar	4.10×10^3	n.g.	n.g.
Garlic	8.74×10^5	4.81×10^4	9.22×10^2
Kiwi	2.24×10^3	3.10×10^3	n.g.
Green onion	3.15×10^5	8.10×10^3	6.24×10^3
Onion	5.95×10^4	1.72×10^3	3.39×10^3
Sesame oil	3.20×10^3	n.g.	n.g.
Black pepper	4.10×10^2	n.g.	n.g.
Total	2.13×10^6	5.90×10^4	1.17×10^4

[a] Detection limit was less than 10^1 (CFU/g).
[b] n.g.: no growth on plate.

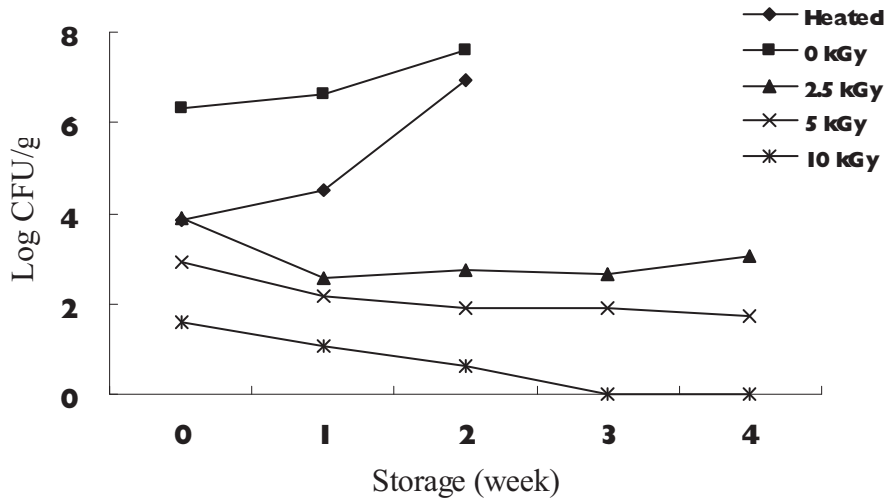

FIG. 1. Growth of Bacillus *spp. in irradiated or heat treated bulgogi sauce samples.*

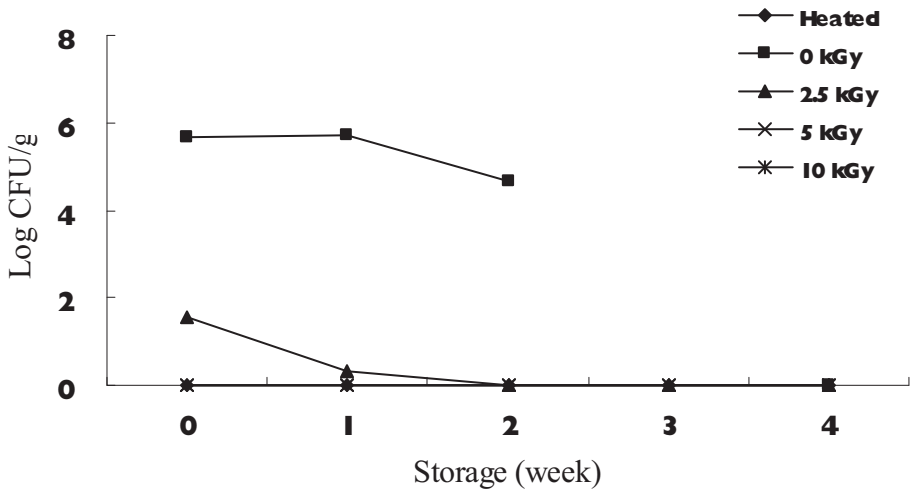

FIG. 2. Growth of coliforms in irradiated or heat treated bulgogi sauce samples.

conducted with these samples after two weeks because of the excessive microbial growth. Irradiation significantly reduced the level of *Bacillus* spp. and this reduced level was maintained during storage.

Song et al. [10] reported that *Bacillus* spp. of Korean type fermented soy sauce, Kanjang, and a Japanese type fermented soy sauce, Shoyu, were reduced by 3 log cycles with 10 kGy, with a D_{10} value for *Bacillus* spp. of 2.67 kGy.

The growth of coliforms in bulgogi sauce samples during the storage period is shown in Fig. 2.

The control sample showed high contamination of coliforms (Fig. 2). Heating at 100°C for 30 min eliminated the coliform bacteria. Sauce irradiated at 2.5 kGy initially had coliforms but these were not found after 2 weeks of storage. Kim et al. [11] reported that the decrease in the microbial population during storage after irradiation was due to the post-irradiation effect, where surviving cells that had been damaged by gamma irradiation gradually died, not having adapted to their surrounding environment during storage. Irradiation at 5 and 10 kGy eliminated coliforms in the bulgogi sauce and no growth was found during storage at 20°C. Lee et al. [12] reported that coliform bacteria were not detected in bulgogi sauce treated by heating the packaging bag at 100°C for 20 min or by gamma irradiation, which is in a good agreement with the present study.

The growth pattern of bacteria in selective media SS agar was similar to coliforms but irradiation even at 2.5 kGy completely controlled the microbial growth.

TABLE 2. pH AND RELATIVE PROTEASE ACTIVITY IN BULGOGI SAUCE IMMEDIATELY AFTER TREATMENT

Treatment	pH	Relative protease activity (%)*
Control**	5.72	100.0a
Heat	5.71	22.3b
2.5 kGy	5.76	102.1a
5.0 kGy	5.75	98.7a
10 kGy	5.76	99.2a
SEM***	0.008	2.3

* Different letters within a column differ significantly ($p < 0.05$).
** Control not heated or irradiated.
*** Pooled standard errors of the mean ($n = 15$).

The pH of the sauce showed no difference between treatments immediately after irradiation (Table 2). Jo et al. [13] reported that the pH of sausage irradiated at 4.5 kGy did not change significantly while rapid reduction in pH was observed in the non-irradiated sausage. The authors interpreted the pH decrease to be due to microbial change, because the acid producing bacterial growth may result in pH reduction.

The relative neutral protease activity of the sauce was significantly reduced by heat treatment, while irradiation had no effect. Heat treatment may denature the proteolytic enzyme, resulting in lower protease activity. Proteolytic enzymes, such as ficin, papain and bromelain, have been used in the tenderizing of meat and act preferentially against connective tissue fibres [14]. Kamphuis et al. [15] reported that actinidin is one of the plant thiol proteinases and is present in kiwi fruit. The scanning electron microscopy of meat treated with crude actinidin showed that it was more fragile than that of the controls. Research has shown that the irradiation of proteins and amino acids in foods has little or no effect on the biological value of the proteins and enzymes are mostly unaffected by low dose irradiation (<1–10 kGy).

Raw bulgogi sauce was also sensorially evaluated (odour and colour) (Table 3). A significant difference was observed in colour at 2.5 kGy, the highest score, while the heat treated sauce was the lowest. Heat treatment of bulgogi sauce resulted in colour changes to the fresh vegetables in the sauce. Major components of the soy sauce are different amino acids and some of these contain sulphur which could affect the odour [16] even though the odour of the soy sauce itself is relatively strong. The sample of heat treated sauce lacked a fresh odour.

TABLE 3. SENSORY EVALUATION OF BULGOGI SAUCE AND COOKED BULGOGI AFTER IRRADIATION* TREATMENT

Sensory parameter	Heat treated	Irradiation dose (kGy)				SEM**
		0	2.5	5.0	10.0	
Bulgogi sauce:						
Odour	4.8	6.3	6.4	6.1	5.2	0.52
Colour***	3.0c	4.9b	7.2a	5.6b	4.9b	0.47
Cooked bulgogi:						
Tenderness	5.8	6.3	6.8	6.0	6.0	0.46
Taste	5.1	6.4	6.1	5.5	5.4	0.56
Acceptance	5.2	6.2	6.1	5.3	5.3	0.52

* Odour and colour were evaluated using a 50 mL sample of sauce. Raw beef was marinated with the sauce at a 2:1 ratio and the marinated beef was placed in a preheated pan at about 170°C and cooked for about 8.5 min to reach approximately 80°C. After cooling for 2 min at room temperature, a 20 g sample was served to the panellists (n = 33) individually. A 9 point hedonic scale was used: like extremely (9) to dislike extremely (1).

** Pooled standard errors of the mean (n = 33).

*** Different letters within the same row differ significantly ($p < 0.05$).

In cooked bulgogi, no statistically significant difference was found but tenderness and taste might be affected by heat treatment of the sauce before cooking.

3.2. Marinated beef ribs

Commercial marinated beef rib samples recorded contamination of 5.68 ln CFU/g of viable cells (Table 4). Pathogens *B. cereus* and *S. aureus* were found at levels of 3.81 and 2.57 ln CFU/g, respectively. These results indicated that even if the marinated beef rib is cooked before consumption, the undercooked meat might pose a potential hazard.

The D_{10} values for bacteria in food are affected by a number of factors, such as water activity, composition, irradiation temperature, presence of oxygen and so on. In addition, some of the constituents of a complex food system, such as proteins, are thought to compete with the cells for interaction with radiolytic free radicals, thereby reducing the net effect of radiation damage and making the organisms sometimes more radiation resistant [17].

TABLE 4. MICROBIAL LOAD IN COMMERCIAL MARINATED BEEF RIBS

Microorganism	Viable cell count (ln CFU/g)
Total aerobic bacteria	5.68 ± 0.03[a]
Coliform bacteria	3.68 ± 0.51
B. cereus	3.81 ± 0.13
S. aureus	2.57 ± 0.20
S. typhimurium	n.v.c.[b]
E. coli	n.v.c.

[a] Mean ± standard deviation ($n = 2$).
[b] n.v.c.: no viable cell growth detected (detection limit was 10^1 CFU/g).

TABLE 5. D_{10} VALUES OF PATHOGENIC BACTERIA IN ARTIFICIALLY CONTAMINATED BEEF RIBS

Microorganism	D_{10} value (kGy) ± SD[a]	r^2
B. cereus	0.66 ± 0.01	0.99
S. aureus	0.59 ± 0.05	0.99
S. typhimurium	0.64 ± 0.02	0.98
E. coli	0.54 ± 0.01	0.99

[a] SD: standard deviation.

Bhide et al. [18] reported that gamma irradiation is not very effective against Gram positive spore forming bacteria such as *B. cereus* and *Clostridium* spp. In fact, the D_{10} value of *B. cereus* in the current study was the highest among the four pathogens.

The D_{10} values observed in marinated beef ribs for *B. cereus*, *S. aureus*, *S. typhimurium* and *E. coli* were calculated to be 0.66 ± 0.01, 0.59 ± 0.05, 0.64 ± 0.02 and 0.54 ± 0.01 kGy, respectively (Table 5). Grant et al. [19] have reported in roast beef meal components D_{10} values in the range 0.252–0.316 kGy and 0.126–0.288 kGy for *S. aureus* and *B. cereus*, respectively, which are lower than the values found in the current study for marinated beef ribs. Thayer and Boyd [20] reported D_{10} values in the range 0.40–0.46 kGy for *S. aureus* in different meat systems irradiated at 5°C.

Viable cells in non-irradiated marinated beef ribs were initially 6 ln CFU/g and doses of 4 kGy presented no viable cell growth of *S. aureus*, *B. cereus*, *S. typhimurium* or *E. coli*. Viable cells of all the four pathogens increased slightly during abusive storage at 20°C (Tables 6–9); *E. coli* was the most radiation sensitive bacteria (Table 9).

TABLE 6. EFFECT OF IRRADIATION ON THE GROWTH OF *S. aureus* IN MARINATED BEEF RIBS DURING STORAGE AT 20°C[a]

Dose (kGy)	Viable cell count (ln CFU/g)			
	0 d	3 d	6 d	9 d
0	6.51 ± 0.60	6.61 ± 0.07	6.22 ± 0.28	6.06 ± 0.04
1	4.99 ± 0.05	5.83 ± 0.18	5.51 ± 0.19	5.32 ± 0.25
2	2.83 ± 0.02	4.32 ± 0.02	4.14 ± 0.51	4.16 ± 0.06
3	1.20 ± 0.28	3.18 ± 0.03	3.88 ± 0.14	3.03 ± 0.01
4	n.v.c.[b]	1.87 ± 0.12	2.25 ± 0.36	2.73 ± 0.09
5	n.v.c.	n.v.c.	1.47 ± 0.12	1.64 ± 0.31

[a] Mean ± standard deviation ($n = 2$).
[b] n.v.c.: no viable cell growth detected (detection limit of 10^1 CFU/g).

TABLE 7. EFFECT OF IRRADIATION ON THE GROWTH OF *B. cereus* IN MARINATED BEEF RIBS DURING STORAGE AT 20°C[a]

Dose (kGy)	Viable cell count (ln CFU/g)			
	0 d	3 d	6 d	9 d
0	6.07 ± 0.06	6.90 ± 0.05	6.71 ± 0.15	6.34 ± 0.19
1	4.74 ± 0.06	5.80 ± 0.03	5.75 ± 0.04	5.70 ± 0.11
2	3.12 ± 0.07	4.28 ± 0.03	4.13 ± 0.01	4.05 ± 0.05
3	1.80 ± 0.07	3.27 ± 0.03	3.17 ± 0.12	3.08 ± 0.22
4	n.v.c.[b]	2.13 ± 0.18	2.15 ± 0.07	2.05 ± 0.07
5	n.v.c.	n.v.c.	1.79 ± 0.21	1.65 ± 0.01

[a] Mean ± standard deviation ($n = 2$).
[b] n.v.c.: no viable cell growth detected (detection limit of 10^1 CFU/g).

TABLE 8. EFFECT OF IRRADIATION ON THE GROWTH OF *S. typhimurium* IN MARINATED BEEF RIBS DURING STORAGE AT 20°C[a]

Dose (kGy)	Viable cell count (ln CFU/g)			
	0 d	3 d	6 d	9 d
0	6.15 ± 0.15	6.21 ± 0.13	6.14 ± 0.09	6.32 ± 0.03
1	5.21 ± 0.06	5.44 ± 0.61	5.89 ± 0.04	5.42 ± 0.23
2	3.91 ± 0.12	4.10 ± 0.10	4.84 ± 0.29	4.34 ± 0.06
3	1.79 ± 0.04	2.43 ± 0.25	3.20 ± 0.08	3.92 ± 0.07
4	n.v.c.[b]	1.89 ± 0.16	2.48 ± 0.20	2.37 ± 0.16
5	n.v.c.	n.v.c.	1.40 ± 0.11	1.35 ± 0.38

[a] Mean ± standard deviation (n = 2).
[b] n.v.c.: no viable cell growth detected (detection limit of 10^1 CFU/g).

TABLE 9. EFFECT OF IRRADIATION ON THE GROWTH OF *E. coli* IN MARINATED BEEF RIBS DURING STORAGE AT 20°C[a]

Dose (kGy)	Viable cell count (ln CFU/g)			
	0 d	3 d	6 d	9 d
0	7.16 ± 0.02	7.40 ± 0.14	6.39 ± 0.12	5.83 ± 0.24
1	5.73 ± 0.04	5.95 ± 0.18	5.27 ± 0.10	4.95 ± 0.07
2	3.12 ± 0.01	4.28 ± 0.19	3.98 ± 0.57	3.83 ± 0.35
3	1.45 ± 0.13	1.96 ± 0.26	2.83 ± 0.24	2.46 ± 0.54
4	n.v.c.[b]	n.v.c.	1.88 ± 0.39	1.87 ± 0.19
5	n.v.c.	n.v.c.	n.v.c.	n.v.c.

[a] Mean ± standard deviation (n = 2).
[b] n.v.c.: no viable cell growth detected (detection limit of 10^1 CFU/g).

After irradiation at 3 kGy, the samples had contamination of about 1 ln CFU/g of viable cells (Tables 10 and 11). However, during commercial conditions (4°C), and after 2 weeks of storage, no viable cell was detected. Sublethal damage to cells caused by irradiation is likely to increase their sensitivity to the environmental stress factors [21]. Similarly, the failure of the radiation injured cells of *S. typhimurium* during refrigerated storage was reported [22]. Patterson [23] pointed out that one of the most common

TABLE 10. EFFECT OF IRRADIATION ON THE GROWTH OF *B. cereus* AND *S. aureus* IN MARINATED BEEF RIBS DURING STORAGE AT 4°C[a]

Dose (kGy)	*B. cereus* (ln CFU/g)			*S. aureus* (ln CFU/g)		
	Initial	2 week	4 week	Initial	2 week	4 week
0	6.07 ± 0.06	6.20 ± 0.17	5.25 ± 0.02	6.51 ± 0.60	6.90 ± 0.02	5.27 ± 0.06
1	4.74 ± 0.06	4.15 ± 0.06	3.55 ± 0.15	4.99 ± 0.05	4.50 ± 0.06	2.54 ± 0.09
2	2.94 ± 0.19	2.33 ± 0.21	1.90 ± 0.08	2.83 ± 0.02	2.17 ± 0.03	1.95 ± 0.07
3	1.52 ± 0.06	n.v.c.[b]	n.v.c.	1.20 ± 0.28	n.v.c.	n.v.c.
4	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
5	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.

[a] Mean ± standard deviation ($n = 2$).
[b] n.v.c.: no viable cell growth detected (detection limit of 10^1 CFU/g).

TABLE 11. EFFECT OF IRRADIATION ON THE GROWTH OF *E. coli* AND *S. typhimurium* IN MARINATED BEEF RIBS DURING STORAGE AT 4°C[a]

Dose (kGy)	*E. coli* (ln CFU/g)			*S. typhimurium* (ln CFU/g)		
	Initial	2 week	4 week	Initial	2 week	4 week
0	7.16 ± 0.02	7.23 ± 0.13	4.83 ± 0.03	6.15 ± 0.15	6.69 ± 0.16	5.55 ± 0.33
1	5.73 ± 0.04	5.64 ± 0.18	2.57 ± 0.38	5.21 ± 0.06	4.53 ± 0.02	3.48 ± 0.15
2	3.12 ± 0.01	2.09 ± 0.12	1.04 ± 0.06	3.91 ± 0.12	2.48 ± 0.09	1.94 ± 0.13
3	1.45 ± 0.13	n.v.c.[b]	n.v.c.	1.79 ± 0.04	n.v.c.	n.v.c.
4	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.
5	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.	n.v.c.

[a] Mean ± standard deviation ($n = 2$).
[b] n.v.c.: no viable cell growth detected (detection limit of 10^1 CFU/g).

combination treatments to obtain a 'hurdle' effect is the use of irradiation with refrigeration. Patterson indicated that a dose of 2.5–3.0 kGy is sufficient to reduce significantly the vegetative pathogens in poultry meat. The combination of storage under refrigeration conditions and 4 and 5 kGy of irradiation eliminated the viable cells of the four pathogens studied (Tables 10 and 11). In studies on the growth of *B. cereus* at 4°C, it has been reported that cells were

unable to grow at pH5–7 and water activity in the range 0.99–0.95 [24]. When fish was inoculated with *B. cereus* and irradiated at 1 and 3 kGy, there was no increase in the number of survivors for weeks at 2–4°C [25]. Recently, Chawla et al. [26] reported that D_{10} values of *B. cereus*, *S. aureus*, *S. typhimurium*, *E. coli* were 0.64, 0.59, 0.56 and 0.55 kGy, respectively, in semi-dried seafood products in the water activity range of 0.90–0.95. In the current study, the raw marinated beef rib has a water activity of 0.99 ± 0.01. Thayer et al. [27] reported that a reduced water content or increased NaCl level may result in the survival levels of the foodborne pathogens of irradiated meat being greater than expected.

3.3. Kimbab

The inhibiting effect of irradiation on *S. typhimurium*, *E. coli*, *S. aureus* and *L. ivanovii,* inoculated into ready-to-eat Kimbab, is illustrated in Tables 12–15. The level of inocula of *S. typhimurium* into the Kimbab was 6.22 ln CFU/g at the initial stage. A dose of 2 kGy reduced the level to 2.29 ln CFU/g and 3 kGy to an undetectable level (Table 12). In fact, Jo et al. [28] had already reported that 3 kGy of irradiation reduced the population of the micro-organism in prepared foods (seasoned and cooked beef, fried egg and ham) to an undetectable level (lower than 10^2 CFU/g).

The effect of irradiation on *E. coli* inoculated into the ready-to-eat Kimbab is shown in Table 13. Irradiation at 2 kGy decreased the *E. coli* level to below 4 ln CFU/g at the beginning of the storage period. These bacteria were not detected after 3 kGy, although they recovered (3 ln CFU/g) after 8–24 h of storage at 20 and 30°C. Lee et al. [29] reported that combined use of irradiation and low temperature storage was effective in attaining microbiological safety of Kimbab and ready-to-use foods.

L. ivanovii inoculated into Kimbab decreased about 4 ln CFU/g with 2 kGy (Table 14), and and was not detected after 3 kGy regardless of the storage temperatures [30, 31]. Kimbab showed a significant reduction with 2 kGy (Table 14). The effect of irradiation on *S. aureus* inoculated into the ready-to-eat Kimbab is shown in Table 15. Kimbab showed a significant reduction with 2 kGy.

Table 16 shows the D_{10} values for different pathogens in artificially contaminated Kimbab samples. The D_{10} values were in the range 0.31–0.44 kGy for the four bacteria studied. Rajkowski et al. [34] found D_{10} values of *E. coli* O157:H7 on broccoli seeds of 1.11–1.43 kGy. Clavero et al. [35] reported that the D_{10} values of *Salmonella* spp. were 0.621–0.624 and 0.618–0.661 on low fat ground beef and high fat ground beef, respectively. The radiation D_{10} values of *L. monocytogenes* on beef were 0.45 kGy at 0°C and 1.21 kGy at –20°C [20]. Lamb et al. [36] reported that two experiments on *S. aureus* in ready-to-eat

TABLE 12. EFFECT OF IRRADIATION ON THE GROWTH OF *S. typhimurium* IN KIMBAB DURING STORAGE AT DIFFERENT STORAGE TEMPERATURES[a]

Storage temperature (°C)	Dose (kGy)	*S. typhimurium* (ln CFU/g)		
		0 h	8 h	24 h
10	0	6.22 ± 0.25	5.88 ± 0.27	5.85 ± 0.21
	1	3.85 ± 0.15	3.88 ± 0.20	3.52 ± 0.35
	2	2.29 ± 0.16	2.09 ± 0.12	2.20 ± 0.28
	3	n.d.[b]	n.d.	n.d.
20	0	6.22 ± 0.25	5.80 ± 0.14	6.26 ± 0.04
	1	3.85 ± 0.15	3.63 ± 0.04	4.06 ± 0.18
	2	2.29 ± 0.16	2.44 ± 0.06	2.57 ± 0.12
	3	n.d.	n.d.	n.d.
30	0	6.22 ± 0.25	5.98 ± 0.19	7.89 ± 0.08
	1	3.85 ± 0.15	4.93 ± 0.76	4.55 ± 0.02
	2	2.29 ± 0.16	3.00 ± 0.14	3.18 ± 0.11
	3	n.d.	n.d.	n.d.

[a] Mean ± standard deviation ($n = 2$).
[b] n.d.: no viable colony growth at detection limit $<10^2$ CFU/g.

TABLE 13. EFFECT OF IRRADIATION ON THE GROWTH OF *E. coli* IN KIMBAB DURING STORAGE AT DIFFERENT STORAGE TEMPERATURES[a]

Storage temperature (°C)	Dose (kGy)	*E. coli* (ln CFU/g)		
		0 h	8 h	24 h
10	0	7.36 ± 0.17	7.33 ± 0.22	7.10 ± 0.11
	1	5.79 ± 0.12	5.81 ± 0.10	5.76 ± 0.16
	2	3.36 ± 0.37	3.27 ± 0.38	2.85 ± 0.21
	3	n.d.[b]	n.d.	n.d.
20	0	7.36 ± 0.17	7.40 ± 0.10	8.78 ± 0.09
	1	5.79 ± 0.12	6.83 ± 0.11	6.93 ± 0.04
	2	3.36 ± 0.37	5.15 ± 0.20	5.00 ± 0.02
	3	n.d.	3.40 ± 0.36	3.84 ± 0.01
30	0	7.36 ± 0.17	8.59 ± 0.06	9.14 ± 0.07
	1	5.79 ± 0.12	7.71 ± 0.01	7.80 ± 0.12
	2	3.36 ± 0.37	4.52 ± 0.39	4.47 ± 0.04
	3	n.d.	3.72 ± 0.18	3.64 ± 0.15

[a] Mean ± standard deviation ($n = 2$).
[b] n.d.: no viable colony growth at detection limit $<10^2$ CFU/g.

TABLE 14. EFFECT OF IRRADIATION ON THE GROWTH OF *L. ivanovii* IN KIMBAB DURING STORAGE AT DIFFERENT STORAGE TEMPERATURES[a]

Storage temperature (°C)	Dose (kGy)	*L. ivanovii* (ln CFU/g)		
		0 h	8 h	24 h
10	0	6.04 ± 0.12	6.94 ± 0.34	7.61 ± 0.37
	1	4.64 ± 0.32	4.46 ± 0.31	4.87 ± 0.06
	2	2.09 ± 0.12	2.09 ± 0.12	2.21 ± 0.05
	3	n.d.[b]	n.d.	n.d.
20	0	6.04 ± 0.12	7.94 ± 0.18	8.61 ± 0.03
	1	4.64 ± 0.32	5.21 ± 0.19	5.08 ± 0.03
	2	2.09 ± 0.12	2.27 ± 0.04	2.46 ± 0.03
	3	n.d.	n.d.	n.d.
30	0	6.04 ± 0.12	8.08 ± 0.01	8.06 ± 0.03
	1	4.64 ± 0.32	5.62 ± 0.29	5.82 ± 0.01
	2	2.09 ± 0.12	2.41 ± 0.09	3.51 ± 0.09
	3	n.d.	n.d.	n.d.

[a] Mean ± standard deviation ($n = 2$).
[b] n.d.: no viable colony growth at detection limit $<10^2$ CFU/g.

TABLE 15. EFFECT OF IRRADIATION ON THE GROWTH OF *S. aureus* IN KIMBAB DURING STORAGE AT DIFFERENT STORAGE TEMPERATURES[a]

Storage temperature (°C)	Dose (kGy)	*S. aureus* (ln CFU/g)		
		0 h	8 h	24 h
10	0	7.87 ± 0.05	7.63 ± 0.09	7.27 ± 0.37
	1	4.11 ± 0.05	4.87 ± 0.24	3.77 ± 0.32
	2	2.09 ± 0.12	2.59 ± 0.16	2.51 ± 0.05
	3	n.d.[b]	n.d.	n.d.
20	0	7.87 ± 0.05	7.65 ± 0.19	8.79 ± 0.01
	1	4.11 ± 0.05	5.81 ± 0.13	5.74 ± 0.31
	2	2.09 ± 0.12	3.80 ± 0.02	4.40 ± 0.11
	3	n.d.	2.80 ± 0.14	2.50 ± 0.14
30	0	7.87 ± 0.05	8.88 ± 0.20	8.81 ± 0.56
	1	4.11 ± 0.05	5.85 ± 0.21	6.26 ± 0.43
	2	2.09 ± 0.12	3.95 ± 0.04	4.96 ± 0.02
	3	n.d.	2.82 ± 0.23	3.09 ± 0.44

[a] Mean ± standard deviation ($n = 2$).
[b] n.d.: no viable colony growth at detection limit $<10^2$ CFU/g.

TABLE 16. D_{10} VALUE OF PATHOGENS IN ARTIFICIALLY CONTAMINATED SAMPLES OF KIMBAB

Pathogen	D_{10} value
S. typhimurium KCTC 1925	0.44 ± 0.01[a]
E. coli KCTC 1682	0.42 ± 0.04
S. aureus KCTC 1916	0.31 ± 0.01
L. ivanovii KCTC 3444	0.43 ± 0.01

[a] Mean ± standard deviation (n = 2).

ham and cheese sandwiches yielded D_{10} values of 0.62 and 0.63. The application of low dose gamma irradiation for the extension of the shelf life, microbiological safety and retention of quality in minimally processed vegetables and fruits has been gaining importance in the industry, including restaurants and institutional and airline catering [34].

Kimchijumeokbab

Kimchijumeokbab, steamed rice with fried kimchi and pork and other food materials, is one of the famous Korean traditional prepared foods taken when people go on a picnic. It is similar to Kimbab. Higher water activity and storage conditions (because of the retrogradation of rice starch) also make this product vulnerable to contamination. The total aerobic bacterial numbers in raw Kimchi mix and raw pork mix were 6.42 and 7.40 ln CFU/g, respectively. The frying procedure presented significant reductions (Table 17). However, lactic acid bacteria must be largely responsible for the higher contamination of raw Kimchi mix. A 10 kGy dose showed no viable cells at day 0 but 2.15 ln CFU/g after 2 d storage at 30°C, which is an abusive temperature condition (Table 18). The viable cells in fried pork and prepared Kimchijumeokbab were not detected in 5 kGy samples at day 0 but were detected at day 1 stored at 30°C.

Sensory evaluation indicated that 3 kGy was the threshhold dose for Kimchijumeokbab (Table 19). It could be concluded that irradiation at 3 kGy combined with low temperature storage improved the safety and ensured the shelf life extension of Kimchijumeokbab without any adverse changes in quality for consumption.

TABLE 17. CONTAMINATION LEVELS IN THE INGREDIENTS FOR KIMCHIJUMEOKBAB

Parameter	Sample	Mean	SEM
Total bacteria counts	Fried Kimchi	4.50	0.05
	Fried pork	2.48	0.01
	Raw Kimchi mix	6.42	0.08
	Raw pork mix	7.40	0.10
Lactic acid bacteria	Fried Kimchi	3.97	0.07
	Raw Kimchi mix	6.55	0.10

TABLE 18. EFFECT OF IRRADIATION ON GROWTH (ln CFU/g) OF TOTAL BACTERIA COUNTS IN FOOD MATERIALS AND KIMCHIJUMEOKBAB DURING STORAGE AT 30°C

Samples	Irradiation dose (kGy)	Storage (day)		
		0	1	2
Fried Kimchi	0	4.50	5.99	8.81
	5	2.56	2.10	4.69
	10	n.v.c.[a]	n.v.c.	2.15
Fried pork	0	2.48	7.44	7.30
	5	n.v.c.	3.62	5.13
	10	n.v.c.	5.09	5.90
Kimchijumeokbab	0	3.94	7.78	8.80
	5	n.v.c.	6.75	8.76
	10	n.v.c.	5.49	8.26
Cooked rice		n.v.c.	n.v.c.	n.v.c.

[a] n.v.c.: no viable cell growth detected.

4. CONCLUSION

The results of these studies indicated that irradiation treatment can minimize the risk of the harmful pathogens in Korean traditional prepared meals including bulgogi sauce, bulgogi, marinated beef ribs, Kimbab and Kimchijumeokbab. Doses of 1–3 kGy were effective in ensuring the safety and

TABLE 19. SENSORY EVALUATION* OF IRRADIATED KIMCHIJUMEOKBAB

Irradiation dose (kGy)	Colour	Flavour	Texture	Taste	Overall acceptance	Odour
0	4.57a	5.36a	5.50a	5.36a	5.86a	2.00b
3	4.00ab	4.86a	4.71ab	4.50ab	5.14ab	2.86b
5	3.86ab	3.43b	3.71bc	3.57bc	3.36c	3.39ab
7	4.36ab	3.43b	4.36bc	3.64bc	4.00bc	3.00ab
10	3.36b	2.93b	3.30c	2.93c	3.14c	4.25a
SEM	0.463	0.536	0.443	0.608	0.594	0.614

* The 7 point hedonic scale was used (n = 25).

extending the shelf life of the prepared foods while maintaining acceptable sensory quality.

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USE OF IRRADIATION TO IMPROVE THE SAFETY AND QUALITY OF ETHNIC SOUTH AFRICAN FOODS

Part I: Combined edible coating and irradiation treatment on sensory and microbiological quality of moist beef biltong

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Abstract

South Africa is a multicultural country with different eating habits and food preferences. Traditional African foods such as bovine tripe form a part of the diet of black South Africans. These foods are laborious to prepare, not generally available commercially, and have a limited shelf life. Other popular ethnic foods in South Africa include meat products such as biltong, an intermediate moisture dried raw meat product. Moist beef biltong has the potential to cause food poisoning. The application of irradiation alone or in combination with other technologies can help solve these problems. Lean moist beef biltong (47% moisture, 3.7% NaCl, 1.5% crude fat, water activity 0.92) can be irradiated with doses up to 10 kGy without adversely affecting sensory acceptability, provided that the irradiation is performed under vacuum conditions and that the biltong is exposed to aerobic conditions after irradiation to dissipate off odour volatiles. However, low dose irradiation (≤4 kGy) was perceived to be more acceptable and preferable by consumers. Gamma irradiation of moist beef biltong (53.6% moisture, 1.91% NaCl, water activity 0.979) at doses between 4 and 5 kGy was adequate to ensure safety from *Staphylococcus aureus* even if contamination levels as high as 10^7 CFU/g were initially present. However, doses up to 5 kGy were insufficient to prevent yeast and mould spoilage if initial fungal contamination levels were high (>10^3 CFU/g). Casein-whey protein edible coatings did not inhibit microbial growth on moist beef biltong, probably owing to diminished oxygen barrier properties resulting from the very high moisture content of the biltong. Ready-to-eat bovine tripe can be irradiated up to 9.3 kGy without affecting the consumer acceptance adversely. Gamma irradiation at a target dose of 9 kGy significantly reduced total bacteria counts and aerobic spore counts and extended the shelf life of ready-to-eat bovine tripe to at least 14 d at both 5 and 15°C when aerobic conditions prevailed. However, this dose may not be sufficient to assure safety of the product if surviving aerobic spores are pathogenic *Bacillus cereus* spores. When anaerobic conditions prevail during processing of

ready-to-eat bovine tripe, the use of boiling in combination with gamma irradiation at a dose of 9 kGy and chilling at 5°C can be used to produce ready-to-eat bovine tripe that is safe from a *Clostridium sporogenes* perspective with an extended shelf life.

South Africa is a multicultural country with different eating habits and food preferences. Traditional African foods such as bovine tripe form a part of the diet of black South Africans. These foods are laborious to prepare, not generally available commercially, and have a limited shelf life. Other popular ethnic foods in South Africa include meat products such as biltong, an intermediate moisture dried raw meat product. Moist beef biltong has the potential to cause food poisoning. The application of irradiation alone or in combination with other technologies can help solve these problems.

The overall objective of the study was to determine the effect of irradiation, alone and in combination with other processing technologies, on the microbiological and sensory quality of moist beef biltong and ready-to-eat bovine tripe, respectively.

1. INTRODUCTION

Beef biltong is nowadays often preferred to be moister than the salty, dry variants of this traditional South African salted, dried, raw meat product. Moist beef biltong (moisture content ≥40%, water activity ≥0.85) [1] can, however, support the growth of the enterotoxin producing pathogen *Staphylococcus aureus* [2]. It also provides an ideal growth medium for spoilage yeasts and moulds [3]. Should significant contamination with these organisms occur, this product could potentially present a health risk and/or have a limited shelf life. Since gamma irradiation is effective in inactivating microorganisms from raw meat products [4] and since milk protein based edible coatings have been proposed in combination with irradiation to inhibit aerobic growth of microbes such as yeasts and mould surviving irradiation by acting as oxygen barriers [5], the effects of these treatments on the microbiological safety and quality of moist beef biltong were determined.

During this research, the effect of gamma irradiation on the sensory perception of moist beef biltong was first established [6]. Thereafter, the effect of a protein based edible coating on inoculated *S. aureus* and the indigenous microbiological flora of whole moist beef biltong was investigated [7].

2. MATERIALS AND METHODS

Experimental designs for this research project are provided in Figs 1 and 2.

Prior to any sensory tests being conducted, representative samples of moist beef biltong were tested for the presence of *S. aureus*, *Salmonella* spp.,

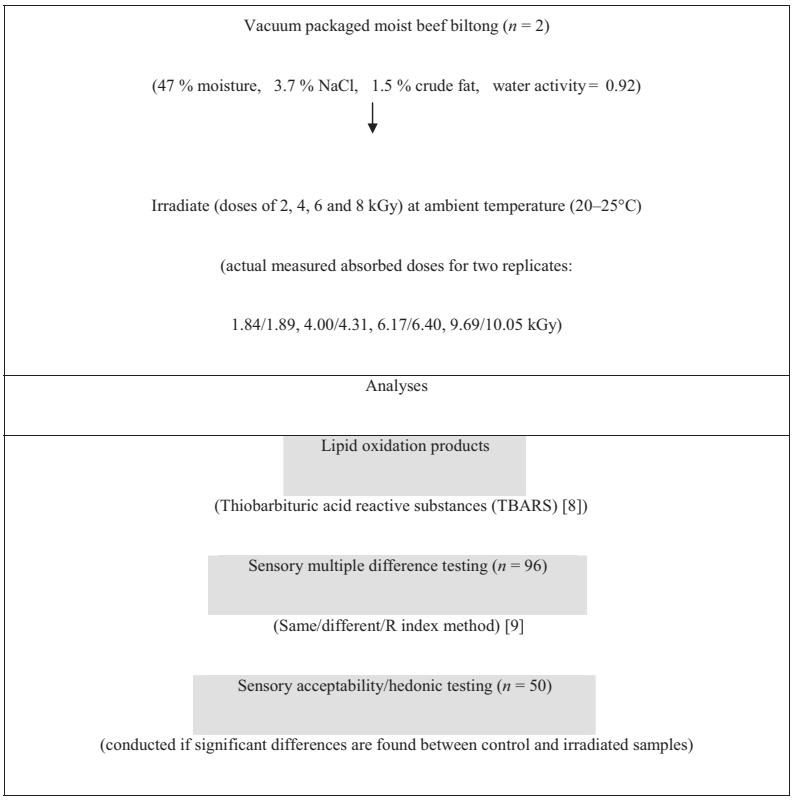

FIG. 1. Experimental design for determination of the effect of irradiation on the sensory perception of moist beef biltong.

Escherichia coli and coliforms. No *E. coli* or *Salmonella* spp. were present. Although total coagulase positive *Staphylococcus* (counts ranging between 7.0×10^2 and 1.4×10^3 CFU/g) were found on all control samples, this was not regarded as holding any health risk since toxin production only commences at cell densities of between 10^5 and 10^6 CFU/g. The samples were therefore considered to be safe for consumption by the sensory panel [6].

An *S. aureus* ATCC 9441 culture was used for challenge testing. Biltong strips were inoculated (10^6–10^7 CFU/g) by spraying 20–25 mL of the inoculum onto each biltong strip with a sterile spray bottle. Control samples were sprayed in the same way with sterile distilled deionized water. All samples were left to dry for 30 min [7].

An edible protein coating solution containing calcium caseinate and whey protein isolate was applied to biltong strips using a spray procedure [7]. Before

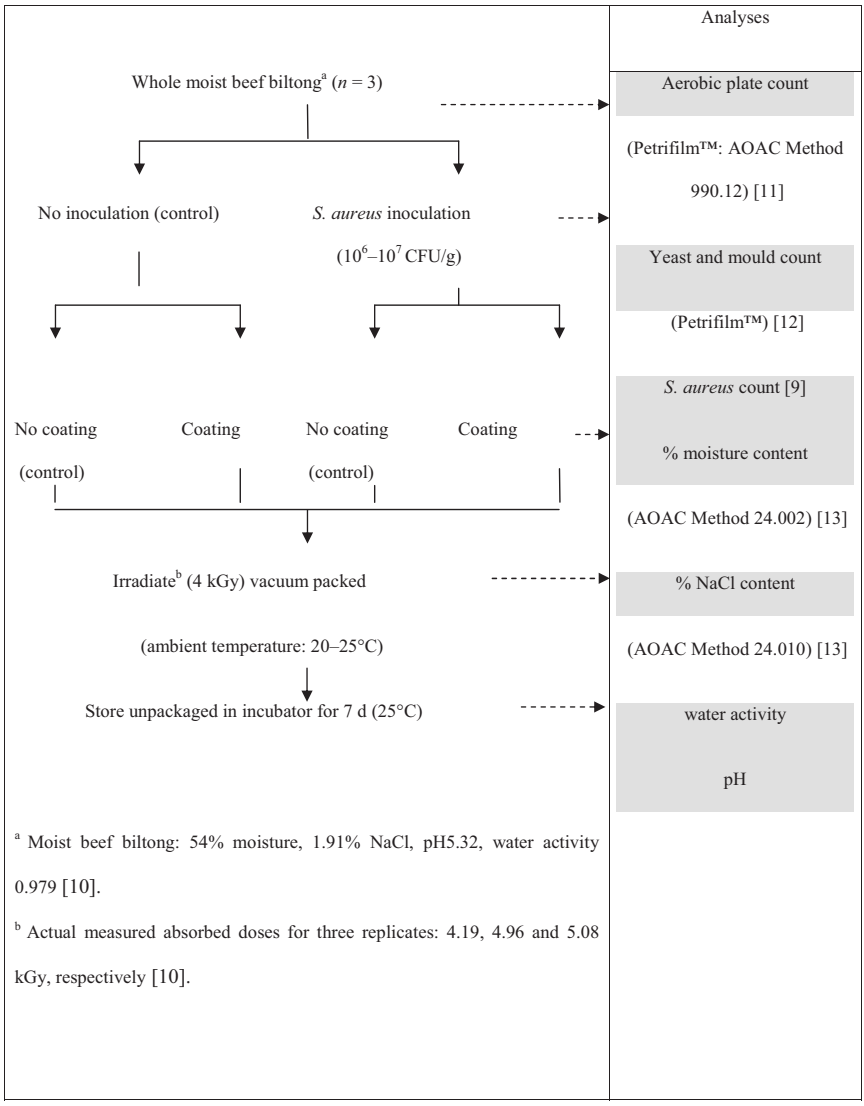

FIG. 2. Experimental design [6, 7] for determination of the effect of irradiation and a casein-whey protein edible coating on the microbiological safety and quality of moist beef biltong. Experiment repeated three times (samples tested in triplicate during individual replicates). Results statistically analysed by ANOVA and Fisher's LSD test.

application, the solution was sterilized at 121°C for 15 min to induce thermal cross-linking to improve water barrier properties [5].

3. RESULTS AND DISCUSSION

3.1. Effect of irradiation on the sensory perception of moist beef biltong [6, 10]

A dose dependent increase in TBARS values was observed (Table 1). However, only biltong irradiated at a dose of approximately 10 kGy (target dose of 8 kGy) had significantly higher levels of oxidation products than untreated biltong, probably due to higher levels of free radicals produced. These results support the findings of other researchers [14–18]. The relatively low TBARS values found in the biltong research were not unexpected given the fact that irradiation processing was done under vacuum conditions and that the fat content of the moist beef biltong was relatively low (i.e. 1.53%). Although Greene and Cumuze [19] found the TBARS threshold level at which unacceptable oxidation flavours were first detected by inexperienced panellists for precooked beef to be between 0.6 and 2 mg/kg, Caballero, Trugo and Finglas [20] stated that TBARS values do not consistently correspond to a specific degree of rancidity in different products. Since a mean TBARS value of approximately 0.9 mg/kg was found for non-irradiated biltong samples, it could be assumed that the threshold for detection of unacceptable flavours in moist beef biltong is in excess of 1.0 mg/kg [6].

TABLE 1. EFFECT OF GAMMA IRRADIATION ON THE TBARS VALUES OF MOIST BEEF BILTONG *(adapted from Ref. [6])*

Irradiation dose[*] (kGy)	Mean TBARS value (± standard deviation)[**] (mg malonaldehyde/kg biltong)
0	0.888 ± 0.054a
2	0.898 ± 0.041a
4	0.929 ± 0.055a
6	0.952 ± 0.062ab
8	1.010 ± 0.062b

[*] Actual measured absorbed doses for two replicates: 2 kGy: 1.84/1.89, 4 kGy: 4.00/4.31, 6 kGy: 6.17/6.40, 8 kGy: 9.69/10.05 kGy.

[**] Mean TBARS values followed by different letters are significantly different ($p < 0.05$).

In contrast to the TBARS analyses, it was found that all irradiated moist beef biltong samples could be distinguished from untreated biltong when sensory difference ratings of an analytical panel were analysed using the R-index method and the Wilcoxon–Mann–Whitney rank sums test. The degree of difference between the irradiated samples was perceived to be rather small, since only ratings of 2 and 10 kGy (target dose of 8 kGy) samples differed significantly ($p < 0.05$) [6].

A consumer acceptability (hedonic) test performed to determine the relation of perceived differences between untreated and irradiated moist beef biltong to the acceptability of the different samples revealed that none of the irradiated biltong samples were significantly less acceptable than the control (0 kGy) (Table 2). However, biltong irradiated at the lower target doses (2 and 4 kGy) was preferred significantly to untreated biltong or biltong irradiated at higher target doses (i.e. 6 and 8 kGy). The preference for low dose irradiated biltong over untreated biltong was unexpected and may be because the slight irradiation flavour that developed in these samples contributed to a fuller, more meaty flavour, since moist beef biltong is usually blander in flavour than drier biltong types due to the higher residual moisture content. This is supported by findings from various researchers [14, 15, 21] where irradiation flavour in beef irradiated at low doses under anaerobic conditions was described as acceptable and meaty, roasted or barbeque-corn-like in nature.

It was therefore concluded that irradiation at a target dose of 4 kGy would be the most suitable irradiation treatment for biltong, since this was the irradiation dose used for which the sensory quality of the biltong was deemed most acceptable and preferable.

TABLE 2. EFFECT OF IRRADIATION ON MEAN HEDONIC RATINGS (± STANDARD DEVIATION) OF MOIST BEEF BILTONG *(adapted from Ref. [6])*

	Dose (kGy)				
	0	2	4	6	8
Overall liking of taste*	5.66 ±2.26a**	7.06 ±1.15b	7.00 ±1.58b	6.02 ±1.71a	5.92 ±1.91a

* Mean hedonic ratings where 1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely.

** Mean hedonic ratings with different letters are significantly different ($p < 0.05$).

3.2. Effect of irradiation and a casein-whey protein based edible coating on the microbiological safety and quality of moist beef biltong [7, 10]

The effect of irradiation and a casein-whey edible coating on the mean *S. aureus*, aerobic plate, yeast and mould counts of inoculated and non-inoculated moist beef biltong samples is summarized in Table 3.

From Table 3, it is clear that considerable variation occurs around the mean microbial counts. Although it is expected that microbial counts would differ somewhat when biltong is produced from different raw materials, and especially during different production runs, as was the case for the three replicates of this experimental work (results not shown but reported [7]), the differences between total bacteria plate, yeast and mould counts of the replicates were highly significant ($p < 0.05$). The reason for this is believed to be

TABLE 3. EFFECT OF IRRADIATION AND A CASEIN-WHEY PROTEIN EDIBLE COATING ON THE MEAN *S. aureus*, AEROBIC PLATE, YEAST AND MOULD COUNTS OF NON-INOCULATED AND INOCULATED MOIST BEEF BILTONG WITH *S. aureus*
(adapted from Ref. [7])

Innoculation with *S. aureus*	Non-irradiated (0 kGy)		Irradiated (4 kGy)	
	Non-coated	Coated	Non-coated	Coated
	Mean *S. aureus* count* (ln CFU/g)**			
Non-inoculated	1.37 ± 1.29b	1.84 ± 1.09b	<1a	<1a
Inoculated	7.21 ± 0.38b	6.96 ± 0.22b	0.73 ± 1.10a	0.67 ± 1.00a
	Mean aerobic plate count (ln CFU/g)			
Non-inoculated	6.06 ± 0.76b	6.23 ± 0.85b	1.44 ± 1.30a	1.01 ± 1.07a
Inoculated	7.21 ± 0.32b	6.99 ± 0.39b	2.04 ± 0.50a	1.76 ± 0.85a
	Mean yeast count (ln CFU/g)			
Non-inoculated	3.50 ± 1.91b	4.03 ± 1.53b	2.14 ± 1.47a	2.06 ± 1.81a
Inoculated	3.27 ± 1.82b	2.94 ± 2.08ab	2.15 ± 1.79ab	1.89 ± 1.62a
	Mean mould count (ln CFU/g)			
Non-inoculated	3.06 ± 2.10b	3.43 ± 1.92b	1.65 ± 1.39a	1.80 ± 1.63a
Inoculated	2.95 ± 1.77b	2.88 ± 2.15b	1.74 ± 1.69a	1.55 ± 1.43a

* Means (± standard deviation) with different letters in a row are significantly different ($p < 0.05$).

** Minimum growth detection limit: 1 ln CFU/g.

due to the implementation of new quality assurance measures by the manufacturer during the research. Strictness of microbiological raw material specifications was increased between production of the first and second biltong replicates. Furthermore, improved drying room cleaning and sanitation processes were implemented between production of the second and third replicates.

Irradiation at a target dose of 4 kGy reduced inoculated *S. aureus* counts by approximately 6 log cycles, irrespective of application of the edible coating. This is not to be unexpected since *S. aureus* is rather sensitive to irradiation. Gamma D_{10} values lower than 0.8 kGy have been reported in various meat products [23–29]. Since viable *S. aureus* counts found on non-inoculated biltong samples never exceeded 10^3 CFU/g, it can be concluded that irradiation at this dose would, under normal conditions, be very effective in rendering moist beef biltong safe from this pathogen. Even in the event of unexpectedly high contamination with *S. aureus* (as in the inoculated samples), the counts are unlikely to exceed 10^6 CFU/g directly after production when irradiation would typically be employed, and a target dose of 4 kGy would thus probably also be effective for elimination in such a case. In contrast, the edible coating had no effect on the level of *S. aureus* when compared with uncoated samples ($p > 0.05$).

Similar results were found for the total bacteria plate counts, irrespective of inoculation with *S. aureus* ATCC 9441 or not. This reduction is highly significant in terms of potential spoilage bacteria such as lactic acid bacteria, which at high counts may cause spoilage of vacuum packaged moist biltong. In addition, the edible coating did not affect ($p > 0.05$) the total bacteria counts significantly.

As was the case for bacteria, the edible coating did not affect ($p > 0.05$) yeast and mould counts significantly. No inhibitory effect on microbial growth was therefore observed for the casein-whey protein coating, and it can thus be concluded that it did not have any significant oxygen barrier properties. A possible reason for this is that the biltong used during the experiments had higher than expected moisture levels (~54% moisture and water activity of 0.98) under which conditions the barrier properties of protein coatings are diminished [30]. Since the effect of the edible coating was determined after 24 h (48 h in conjunction with irradiation), it may be that the time period was not long enough for a significant effect to be observed. For this reason, it was planned to test samples after 7 d of storage. Owing to gross fungal spoilage in less than 48 h under the specific storage conditions used, these samples were deemed unfit for human consumption and consequently not tested. No visual difference between coated and non-coated samples could be seen, but it is not

known whether it would have had any effect if lower numbers of microbes survived irradiation and/or if less favourable conditions were employed.

As anticipated, irradiation caused a significant reduction ($p < 0.05$) in yeast and mould counts. However, because fungi are more resistant to irradiation than bacteria [31], a relatively smaller reduction in counts (1–2 log cycles) compared with that of bacteria (i.e. 6 log cycles) was observed. When good manufacturing practices are used during manufacture of biltong, levels of yeasts and moulds are not expected to exceed 100–1000 CFU/g (as found for the biltong samples during the third replicate of the experiment) [7]. Therefore, reductions in fungal counts by 1–2 log cycles due to irradiation at doses between 4 and 5 kGy could extend the shelf life of moist beef biltong significantly under these circumstances. However, in the biltong samples tested during the first two repetitions, yeast and mould counts of up to 10^4–10^5 CFU/g were found [7]. Irradiation at between 4 and 5 kGy would clearly not be adequate in this case to prevent microbial spoilage due to surviving yeasts and moulds (i.e. 10^3–10^4 CFU/g).

4. CONCLUSIONS AND RECOMMENDATIONS

Gamma irradiation at doses of between 4 and 5 kGy will be adequate to ensure the safety of moist beef biltong in terms of *S. aureus*. This dose will not, however, be effective in controlling fungal growth in moist beef biltong if initial contamination levels are high ($\geq 10^4$ CFU/g) and conditions are favourable for fungal growth. It is therefore clear that strict hygienic practices and microbiological standards must be adhered to during and after the manufacture of moist biltong, since irradiation up to 5 kGy will not be effective in eliminating high fungal loads.

Since it was found that irradiation at doses up to 10 kGy did not detrimentally affect the sensory quality of moist beef biltong, irradiation at higher doses may potentially be used to control yeast and mould growth on moist biltong. Otherwise, the use of a combination of ‘hurdles’ (e.g. vacuum packaging and protein coatings with incorporated preservatives), together with low dose irradiation (≤5 kGy), may be employed to attain extended microbiological stability.

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USE OF IRRADIATION TO IMPROVE THE SAFETY AND QUALITY OF ETHNIC SOUTH AFRICAN FOODS

Part II: Effect of gamma irradiation on the sensory and microbiological quality of ready-to-eat bovine tripe

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Abstract

South Africa is a multicultural country with different eating habits and food preferences. Traditional African foods such as bovine tripe form a part of the diet of black South Africans. These foods are laborious to prepare, not generally available commercially, and have a limited shelf life. Other popular ethnic foods in South Africa include meat products such as biltong, an intermediate moisture dried raw meat product. Moist beef biltong has the potential to cause food poisoning. The application of irradiation alone or in combination with other technologies can help solve these problems. Lean moist beef biltong (47% moisture, 3.7% NaCl, 1.5% crude fat, water activity 0.92) can be irradiated with doses up to 10 kGy without adversely affecting sensory acceptability, provided that the irradiation is performed under vacuum conditions and that the biltong is exposed to aerobic conditions after irradiation to dissipate off odour volatiles. However, low dose irradiation (≤4 kGy) was perceived to be more acceptable and preferable by consumers. Gamma irradiation of moist beef biltong (53.6% moisture, 1.91% NaCl, water activity 0.979) at doses between 4 and 5 kGy was adequate to ensure safety from *Staphylococcus aureus* even if contamination levels as high as 10^7 CFU/g were initially present. However, doses up to 5 kGy were insufficient to prevent yeast and mould spoilage if initial fungal contamination levels were high (>10^3 CFU/g). Casein-whey protein edible coatings did not inhibit microbial growth on moist beef biltong, probably owing to diminished oxygen barrier properties resulting from the very high moisture content of the biltong. Ready-to-eat bovine tripe can be irradiated up to 9.3 kGy without affecting the consumer acceptance adversely. Gamma irradiation at a target dose of 9 kGy significantly reduced total bacteria counts and aerobic spore counts and extended the shelf life of ready-to-eat bovine tripe to at least 14 d at both 5 and 15°C when aerobic conditions prevailed. However, this dose may not be sufficient to assure safety of the product if surviving aerobic spores are pathogenic *Bacillus cereus* spores. When anaerobic conditions prevail during processing of ready-to-eat bovine tripe, the use of boiling in

combination with gamma irradiation at a dose of 9 kGy and chilling at 5°C can be used to produce ready-to-eat bovine tripe that is safe from a *Clostridium sporogenes* perspective with an extended shelf life.

1. INTRODUCTION

Bovine tripe is not optimally used in South Africa because it is highly perishable [1], not readily available in a convenient form and it requires long cooking time [2]. For these reasons, ready-to-eat technology was used to process tripe. One organism of particular concern in cook–chill foods is *Clostridium perfringens.* Furthermore, this organism has been found on intestinal tract offal [3, 4]. The current research was undertaken to determine the effect of vacuum packaging and boiling of tripe in combination with gamma irradiation on the microbiological safety, with respect to inoculate *C. perfringens* ATCC 13124 spores, and microbiological quality of ready-to-eat bovine tripe.

2. MATERIALS AND METHODS

The initial intention was to inoculate ready-to-eat bovine tripe and process it as a sous vide product in an anaerobic environment (vacuum packaged). This is shown in the experimental design provided (Experiment 1, Fig. 1). However, no *C. perfringens* was detected in inoculated samples after boiling or during storage as a result of the presence of oxygen in the system. Consequently, a second experiment was performed where raw tripe was first boiled, then chilled and inoculated prior to vacuum packaging and gamma irradiation. Since a shelf-life study (14 d) was conducted in Experiment 1, the shelf life study in Experiment 2 was shortened to 7 d. Figure 2 illustrates the experimental design for Experiment 2.

However, before any microbiological studies were conducted it was important to determine the effect that gamma irradiation would have on consumer sensory acceptance of ready-to-eat bovine tripe.

2.1. Effect of gamma irradiation on consumer sensory acceptance of ready-to-eat bovine tripe

Fresh, roughly washed bovine tripe (i.e. rumen, reticulum and omasum) was cleaned further by washing thoroughly with 21ºC tap water. It was then

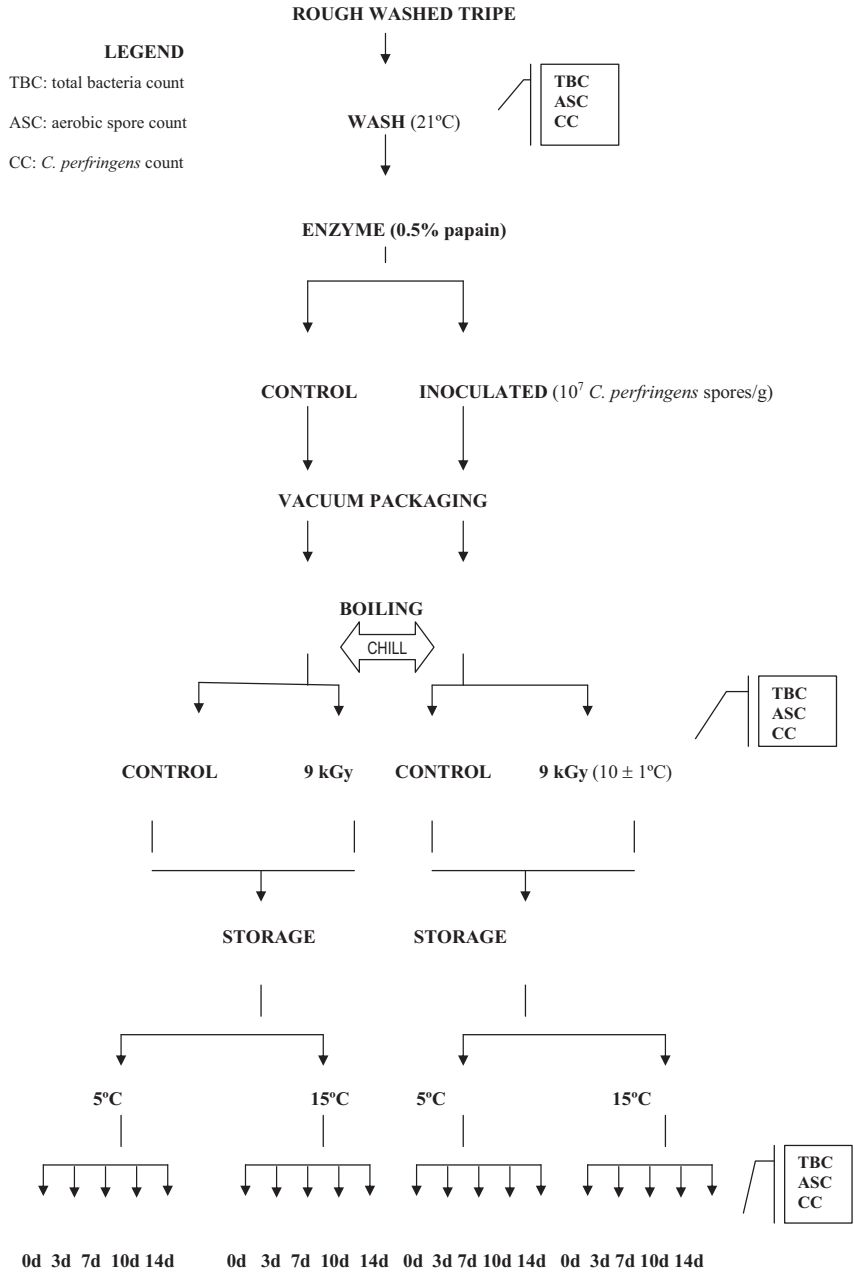

FIG. 1. Experimental design for determination of the effect of boiling and gamma irradiation at a dose of 9 kGy on the bacterial quality of ready-to-eat bovine tripe stored for 14 d at 5 and 15°C [4].

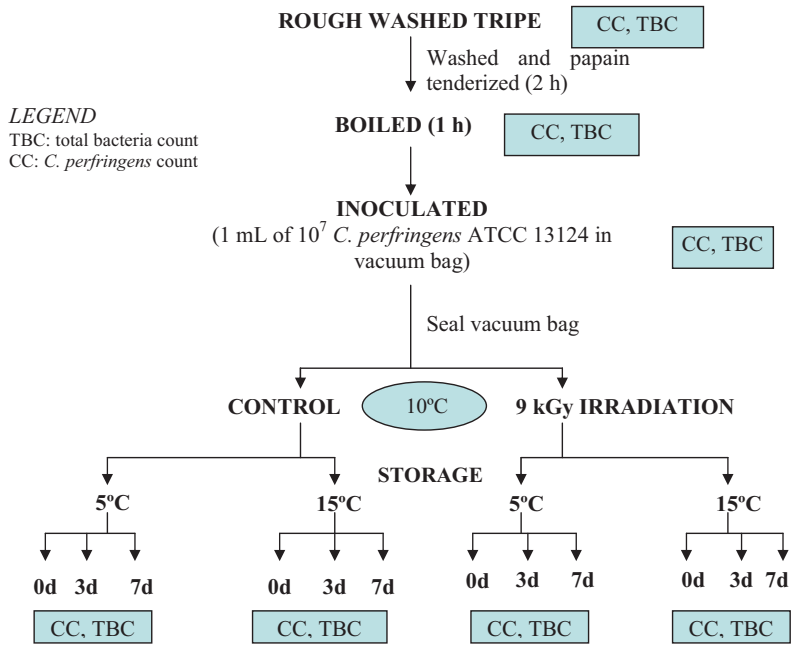

FIG. 2. Experimental design to determine the effect of gamma irradiation at a target dose of 9 kGy on survival of C. perfringens *ATCC 13124 spores on ready-to-eat bovine tripe stored at 5 and 15°C for 7 d [4].*

partially defatted before cutting into 4 cm × 4 cm pieces. Tripe was tenderized by soaking the pieces in a 0.5% (w/v) solution of papain (pH4.5) at room temperature for 2 h. After enzyme treatment, one hour of cooking was required to produce a ready-to-eat product. After cooking, the samples were vacuum packaged and irradiated to target doses of 2, 4, 6 and 8 kGy using ^{60}Co gamma rays at Isotron SA (Isando, South Africa). The actual doses received were 2.6, 5.0, 6.4 and 9.3 kGy, respectively.

2.1.1. Microbiological analyses

To ensure that only samples fit for human consumption would be provided to the consumer panel, total plate counts and aerobic spore counts (ASC) were conducted on samples irradiated to target doses of 2, 4, 6 and 8 kGy.

2.1.2. Consumer sensory evaluation

The 9 point hedonic scale (1 = dislike extremely, 9 = like extremely) was used to evaluate the appearance, texture, flavour and overall acceptability of the tripe samples. A total of 51 consumers participated in the evaluations. The majority of the consumers were older than 30 years of age and 69% of the panel were black South Africans. More males (69%) than females (31%) participated in the evaluation.

2.2. Effect of boiling and gamma irradiation on bacterial quality and *C. perfringens* ATCC 13124 spores inoculated on ready-to-eat bovine tripe, and the shelf life of ready-to-eat bovine tripe stored at 5 and 15ºC for 14 d [3]

In Experiment 1, fresh tripe was processed as a sous vide product. Washed tripe was inoculated with 7 ln CFU/g *C. perfringens* ATCC 13124 spores in bags, vacuum sealed, boiled in bags for 1 h and irradiated with a dose of 9 kGy (actual doses: 9.2, 9.0 and 7.9 kGy). Aerobic conditions prevailed owing to the presence of residual oxygen in the packs resulting in the inhibition of *C. perfringens* after boiling. Preparation of ready-to-eat bovine tripe took 1 d and the irradiation treatment took 2 d. Therefore, storage days 0, 3, 7, 10 and 14 at 5 and 15°C were actually days 3, 6, 10, 13 and 17. In Experiment 2, bovine tripe was boiled for 1 h prior to inoculation with spores, vacuum packaged and irradiated with a target dose of 9 kGy (actual doses: 8.4 and 8.9 kGy). Preparation of ready-to-eat bovine tripe also took 1 d, but the irradiation treatment took 3 d. Therefore storage days 0, 3 and 7 were actually days 4, 7 and 11 at 5 and 15°C after preparation, respectively.

For both experiments, total bacteria counts (TBC), ASC and *C. perfringens* counts were done on the following samples: roughly washed bovine tripe, papain tenderized samples, after boiling, immediately after gamma irradiation and at different time intervals during storage at 5 and 15°C. The *C. perfringens* spore count was made after heat shocking the samples at 70ºC for 20 min. Optical microscopy was also used to confirm that spores were counted and not vegetative cells.

To study the potential synergistic effect of boiling in combination with gamma irradiation, transmission electron microscopy was conducted on pure *C. perfringens* ATCC 13124 spores as follows: untreated, boiled alone for 1 h at 100°C, irradiated alone at 9 kGy, boiled in combination with irradiation treatment.

3. RESULTS AND DISCUSSION

3.1. Effect of gamma irradiation on consumer sensory acceptance of cooked ready-to-eat bovine tripe

3.1.1. Microbiological assays

The TBC (Fig. 3) of the uncooked tripe was high (i.e. 6.27 ln CFU/g), but as expected for fresh tripe [1–5]. There was only a slight reduction in TBC after cooking (0 kGy) to 6.02 ln CFU/g. This may be due to the fact that a large part of the TBC consisted of aerobic spore formers. As the irradiation dose increased, both the TBC (Fig. 3) and aerobic spore former counts (Fig. 4) decreased. A 3 ln CFU/g reduction was evident with the samples irradiated to a target dose of 8 kGy in both counts. No *C. perfringens* was detected in the samples, either before or after irradiation. Therefore, regarding *C. perfringens,* the ready-to-eat tripe was considered to be safe. However, the results indicated that if aerobic spore formers comprise a large component of the TBC, the irradiation dose necessary to reduce the count to an acceptable level may have to be equal to or exceed 6 kGy. Subsequently, a target dose of 9 kGy was used for irradiation of ready-to-eat bovine tripe.

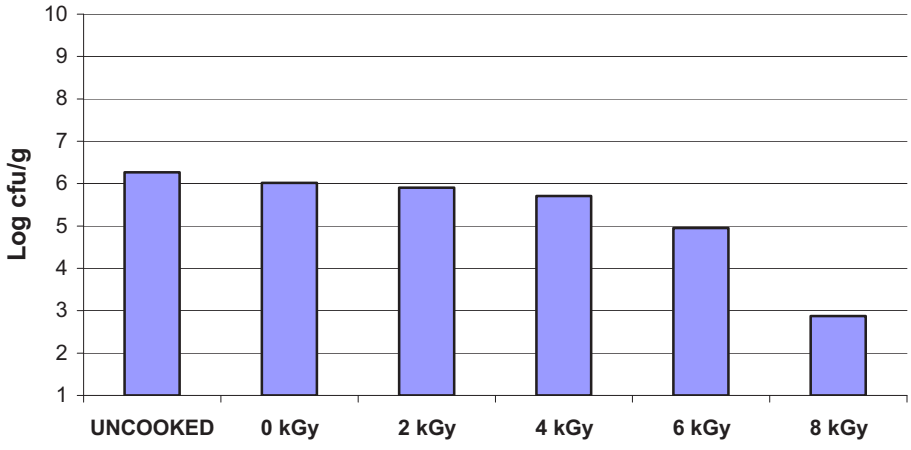

FIG. 3. TBC of uncooked (unirradiated) versus cooked ready-to-eat bovine tripe after irradiation at doses of 2, 4, 6 and 8 kGy.

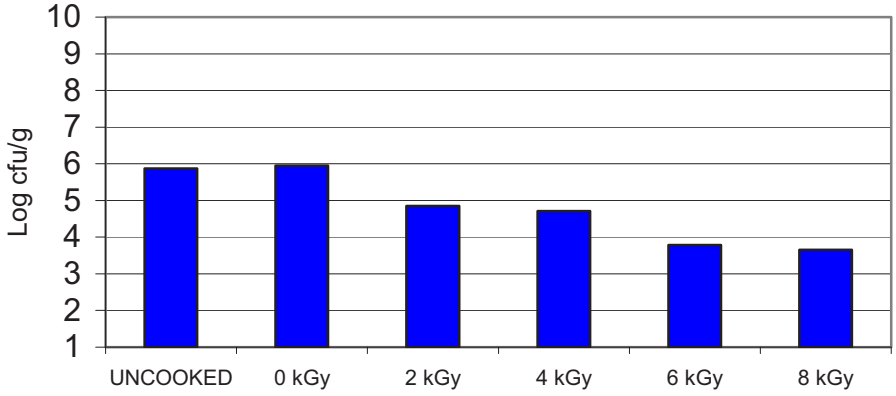

FIG. 4. Effect of gamma irradiation on ASC of ready-to-eat bovine tripe.

3.1.2. Consumer sensory evaluation

There were no significant differences in the mean hedonic ratings for the appearance, aroma, texture, flavour and overall acceptability ($p > 0.05$) between irradiated and control tripe samples (Table 1).

The mean rating scores were quite low, i.e. dislike slightly to neither liked nor disliked. The standard deviations were also quite large. The reason for this became evident after cluster analyses were conducted. It was clear that the majority of panellists (black consumers) gave relatively high ratings for all samples, whereas the minority of panellists (white, coloured and Asian consumers) gave relatively low scores.

The majority of the consumers (59–74%) gave positive response ratings (above 6 on the 9 point scale) for all of the attributes evaluated, while the rest of the consumers were more negative. The negative results could be the result of the toughness of the tripe as well as a bland flavour. Not every consumer was used to consuming tripe on its own, without a sauce or other food items added. This might also have had an influence on the negative responses of some of the consumers. This was probably due to the fact that the samples were prepared in a way familiar to African consumers but not necessarily familiar to the other ethnic groupings. In fact, some white consumers declined to evaluate the samples when they saw that they were not prepared in the way that they were used to.

TABLE 1. EFFECT OF IRRADIATION ON THE AVERAGE HEDONIC RATINGS (± STANDARD DEVIATION) OF COOKED, READY-TO-EAT BOVINE TRIPE

Sensory characteristics	Dose (kGy)[a]					*p* value
	0	2	4	6	8	
Look/appearance	5.43 ± 2.73[b]	5.65 ± 2.64	5.54 ± 2.64	5.85 ± 2.44	5.77 ± 2.74	0.94
Smell/aroma	5.56 ± 2.37	5.59 ± 2.33	5.73 ± 2.24	5.63 ± 2.24	5.90 ± 2.43	0.96
Texture	4.80 ± 2.64	5.20 ± 2.51	5.02 ± 2.45	5.23 ± 2.41	5.69 ± 2.64	0.54
Flavour/taste	4.67 ± 2.37	5.04 ± 2.47	4.94 ± 2.35	4.81 ± 2.58	5.25 ± 2.69	0.83
Overall acceptability	4.79 ± 2.46	5.06 ± 2.55	4.85 ± 2.58	5.06 ± 2.56	5.37 ± 2.50	0.82

[a] Actual irradiation doses: 2.6, 5.0, 6.4 and 9.3 kGy.
[b] Mean hedonic ratings where: 1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely.

3.2. Effect of boiling and gamma irradiation on bacterial quality and *C. perfringens* ATCC 13124 spores inoculated on ready-to-eat bovine tripe, and on the shelf life of ready-to-eat bovine tripe stored at 5 and 15°C for 14 d

3.2.1. Experiment 1: Aerobic conditions prevailing

As expected, the raw bovine tripe had high microbiological loads for TBC and ASC as well as relatively high levels of *C. perfringens* (Table 2). This is due to the high levels of microorganisms naturally present in the ruminant stomach. Although boiling for 1 h reduced both TBC and ASC, this was not statistically significant. Furthermore, these levels remained high after boiling, probably due to the presence of heat resistant aerobic spore formers [6]. This is in agreement with studies by researchers [1, 6] who reported the presence of heat resistant *Deinococcus* and lactobacillus on cooked bovine tripe and heat resistant *Aeromonas* spp., *Clostridium sporogenes* and *Bacillus* spp. on minced tripe.

Overall, gamma irradiation had a statistically significant ($p = 0.017$) effect on the TBC of ready-to-eat bovine tripe. There were, however, no significant effects between storage temperature and storage time on the TBC of ready-to-eat bovine tripe samples (Fig. 5). Similarly, gamma irradiation overall significantly ($p = 0.014$) reduced the TBC on ready-to-eat bovine tripe. Again, there were no significant storage temperature or time effects or any significant interactions between the variables (Fig. 6).

TABLE 2. EFFECT OF ROUGH WASHING, PAPAIN TREATMENT AND BOILING ON THE BACTERIAL QUALITY (± STANDARD DEVIATION) OF FRESH BOVINE TRIPE (n = 18) *(adapted from Ref. [3])*

Treatment	TBC	ASC	*C. perfringens* count
Rough washed	8.6 ± 1.6a*	8.6 ± 1.6a	4.5 ± 1.2a
Papain tenderized	8.8 ± 0.7a	8.6 ± 1.0a	2.2 ± 2.0a
Boiled	6.3 ± 0.4a	6.1 ± 0.7a	2.0 ± 2.2a

* Mean values followed by a different letter within the same column are significantly different from each other ($p < 0.05$).

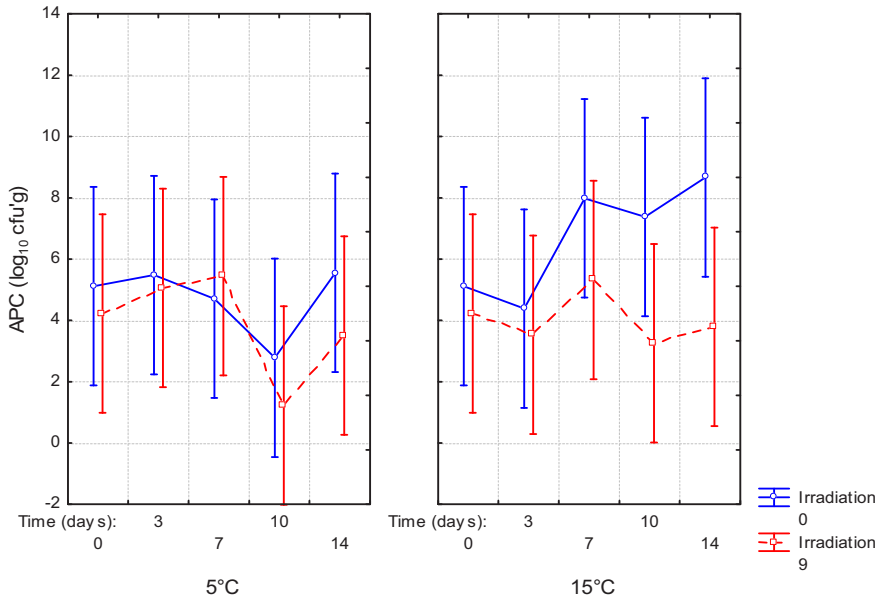

FIG. 5. Effect of irradiation at a target dose of 9 kGy on TBC of ready-to-eat bovine tripe stored for 14 d at 5 and 15°C (adapted from Ref. [3]).

Gamma irradiation caused a significant reduction in TBC (Fig. 5) and ASC (Fig. 6) on ready-to-eat bovine tripe. The levels of TBC and ASC overall, after irradiation, were 4 ln CFU/g and 3.9 ln CFU/g, respectively. These levels suggest that the aerobic bacteria and aerobic spores on tripe show high resistance to boiling and irradiation. Although most of the enteric bacteria found on fresh tripe are sensitive to low doses of ionizing irradiation (~1 kGy), irradiation resistant *Deinococcus*, *Aeromonas*, *Staphylococcus* and *Streptococcus*

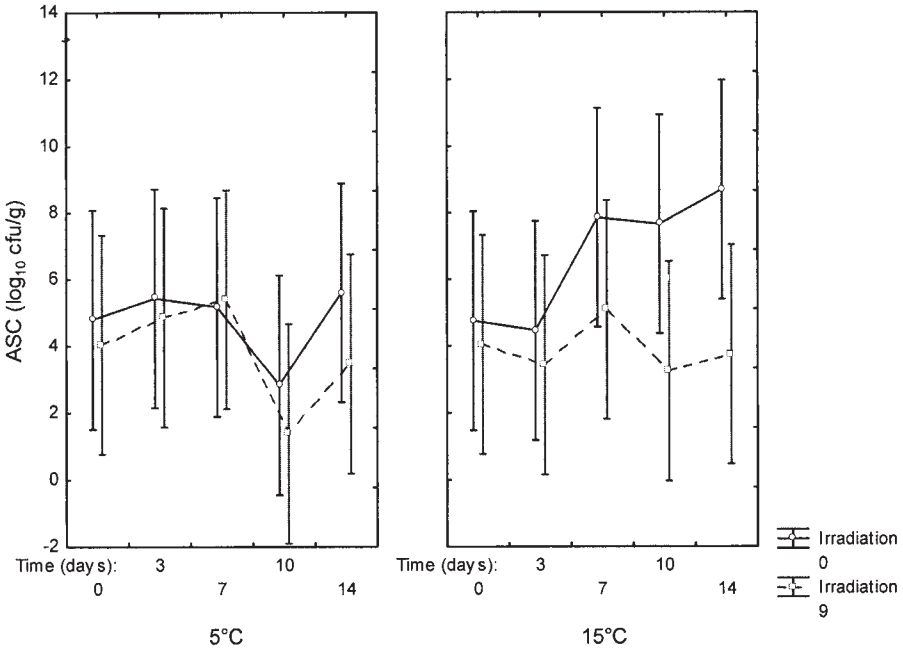

FIG. 6. Effect of irradiation at a target dose of 9 kGy on ASC of ready-to-eat bovine tripe stored for 14 d at 5 and 15°C (adapted from Ref. [3]).

have been reported on tripe [1, 7–9]. It is also postulated that most of the aerobic spores on ready-to-eat bovine tripe belong to the genus *Bacillus* [2]. An identical pattern of growth was observed for both TBC and ASC and 0 and 9 kGy on ready-to-eat bovine tripe stored for 14 d at 5 and 15°C. For this reason, as well as the high resistance of APC to boiling (95°C) and gamma irradiation, it is postulated that the TBC reported in this study were predominantly aerobic spores.

Interestingly, the TBC and ASC on 9 kGy ready-to-eat bovine tripe stored at 15°C also followed the same trend as the 0 and 9 kGy samples stored at 5°C. The TBC and ASC of the 0 kGy ready-to-eat bovine tripe samples stored at 15°C increased progressively until they reached unacceptable levels of 8.6 ln CFU/g and 8.8 ln CFU/g, respectively, by day 14.

It has been reported that processed and vacuum packaged tripe showed signs of spoilage when its bacterial numbers reached 7 ln CFU/g [8]. Growth of TBC and ASC on 0 and 9 kGy ready-to-eat bovine tripe was restricted at 5°C with TBC and ASC remaining below 6 ln CFU/g throughout storage, thus extending the shelf life to at least 14 d. Therefore, storage of ready-to-eat bovine tripe, packed under aerobic conditions, at 5°C is sufficient to prevent

germination and rapid proliferation of aerobic bacteria and aerobic spores. Even when the initial ASC is high, they can be contained at this refrigerated temperature.

Similarly, TBC and ASC on irradiated ready-to-eat bovine tripe samples stored at 15°C were contained around 4 ln CFU/g throughout 14 d of storage. Although this temperature could be conducive for repair and proliferation, the increase in APC and ASC was controlled. This is probably because gamma irradiation induced injuries to aerobic bacteria and aerobic spores in such a way that they were unable to germinate and grow at 15°C. This suggests that gamma irradiation at a target dose of 9 kGy is sufficient to prolong the shelf life of ready-to-eat bovine tripe to at least 14 d, even at an abusive temperature of 15°C. Similarly, it has been reported [7] that irradiation of beef sauce at 5 and 10 kGy reduced the microbiological counts of thermophilic bacteria, whose numbers remained low throughout the 4 weeks of storage at 20°C. However, unlike irradiated samples stored at 15°C, non-irradiated samples stored at 15°C showed a steady increase in spore numbers. By day 7, ASC had exceeded 7 ln CFU/g and thus by day 7, the non-irradiated samples stored at 15°C were considered to be spoiled.

3.2.2. Experiment 2: Anaerobic conditions prevailing

As with Experiment 1, the raw bovine tripe recorded a very high TBC (8.4 ln CFU/g), which was significantly reduced by gamma irradiation. This was expected since most of the enteric bacteria on tripe are sensitive to low doses of gamma irradiation [1].

Overall, gamma irradiation had a statistically significant ($p = 0.000$) effect on the TBC of inoculated ready-to-eat bovine tripe as well as on *C. perfringens* ATCC 13124 spores. There were, however, no significant effects between storage temperature and storage time on the TBC of ready-to-eat bovine tripe samples (Table 3). There were no significant storage temperature or storage time effects or interactions in terms of survival of *C. perfringens* ATCC 13124.

Initially, no growth of aerobic bacteria was detected on irradiated samples stored at 5 and 15°C. However, during storage at both 5 and 15°C, aerobic bacteria re-emerged on irradiated samples probably due to repair of irradiation injury. A lag phase was observed before regrowth occurred.

Transmission electron microscopy of isolated *C. perfringens* ATCC 13124 spores showed that boiling alone caused reduction of spore material possibly due to initiation of germination. Gamma irradiation alone caused elongation of *C. perfringens* spores indicating that outgrowth was inhibited even though germination occurred [4]. The heat–irradiation combination had a synergistic

TABLE 3. EFFECT OF IRRADIATION ON TBC OF INOCULATED READY-TO-EAT BOVINE TRIPE AND ON SURVIVAL OF *C. perfringens* ATCC 13124 SPORES ON INOCULATED READY-TO-EAT BOVINE TRIPE STORED FOR 7 d AT 5°C AND 15°C *(adapted from Ref. [3])*

Storage time (d)	TBC (ln CFU/g)		*C. perfringens* ATCC 13124 (ln CFU/g)	
	Irradiation dose (kGy)*			
	0	9	0	9
5°C				
0	10.13 ± 0.57	<1**	6.09 ± 0.16	<1
3	9.02 ± 0.29	<1	6.65 ± 0.73	<1
7	10.07 ± 0.07	1.41 ± 1.99	5.54 ± 1.02	<1
15°C				<1
0	10.13 ± 1.20	<1	6.09 ± 0.16	<1
3	9.88 ± 0.29	1.88 ± 2.70	6.70 ± 1.02	<1
7	10.43 ± 0.37	1.91 ± 2.70	6.81 ± 0.96	<1a
Overall irradiation effect***	10.0b	1.0a	6.4b	<1a

* Actual irradiation doses: 8.4 kGy and 8.9 kGy for replicates 1 and 2.
** Minimum growth detection limit: 1 ln CFU/g.
*** Mean values followed by a different letter within the same cell are significantly different from each other ($p < 0.05$).

effect on *C. perfringens* spores as indicated by the complete loss of spore material [4].

Under the anaerobic conditions prevailing in Experiment 2, inoculated *C. perfringens* ATCC 13124 spores were eliminated by gamma irradiation. This is in agreement with Van den Heever [1], who reported that irradiation at 4–6 kGy is sufficient to reduce 6 ln CFU/g *C. perfringens* below levels that are easily recoverable by conventional bacteriological methods. Gamma D_{10} values for *C. perfringens* have been reported to range between 1.2 and 3.4 kGy [8]. Furthermore, no *C. perfringens* was detected on irradiated ready-to-eat bovine tripe samples stored at both 5 and 15°C throughout the 7 d of storage. Parry-Hanson [4] proposed that the injury to the post-germination system of *C. perfringens* ATCC 13124 was permanent, thus not allowing for spore injury repair during the storage period.

4. CONCLUSIONS

Consumer sensory evaluation indicated that gamma irradiation up to 9.3 kGy did not have an adverse effect on acceptability of ready-to-eat bovine tripe. However, the results indicated that fresh tripe has high TBC and ASC, making it an ideal product for irradiation. Therefore, an irradiation dose equal to or exceeding 6 kGy would be necessary to reduce the microbial contamination to acceptable levels in order to attain an acceptable shelf life.

Gamma irradiation at a target dose of 9 kGy is sufficient to prolong the shelf life of ready-to-eat bovine tripe to at least 14 d storage under aerobic conditions, even at the abusive temperature of 15°C. However, owing to the high levels of aerobic spores and aerobic bacteria that persisted after irradiation processing, sous vide processing of bovine tripe is not recommended because it might be unsafe for consumption if pathogenic *B. cereus* spores are present. Irradiated ready-to-eat bovine tripe prepared in an anaerobic environment proved to be safe for human consumption from a *C. perfringens* point of view for at least 7 d of storage, even under abusive storage temperatures (i.e. 15°C).

ACKNOWLEDGEMENTS

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EFFECT OF GAMMA IRRADIATION ON THE MICROBIAL LOAD AND CHEMICAL AND SENSORY PROPERTIES OF LOCALLY PREPARED FAST MEALS

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Syrian Arab Republic

Abstract

Locally prepared meals (kubba, borak, cheese borak and sheesh tawoq) were treated with 2, 4 or 6 kGy doses of gamma irradiation. Treated and untreated samples were kept in a refrigerator (1–4°C). Microbiological and chemical analyses were performed on each treated sample immediately after processing, and weekly throughout the storage period, which lasted for 3 weeks for kubba, 6 weeks for borak and cheese borak and 20 weeks for sheesh tawoq. Sensory evaluation and proximate analysis were done within one week after irradiation. Results of the proximate analysis of borak, cheese borak and sheesh tawoq showed that the irradiation doses did not have a significant effect on the moisture, protein and fat contents of meals, whereas for kubba, irradiation decreased the moisture, protein and fat contents. The doses of gamma irradiation selected decreased the microorganism load and increased the shelf life of all meals studied. The radiation doses required to reduce *Salmonella* and *Escherichia coli* by one log cycle (D_{10}) in borak were 0.46 and 0.51 kGy, in cheese borak 0.30 and 0.50 kGy and for sheesh tawoq 0.44 and 0.39 kGy, respectively. The three chemical parameters, total acidity, lipid peroxide and volatile basic nitrogen, which were chosen as the indices of freshness, were all well within the acceptable limit for up to 3 weeks for kubba, 6 weeks for borak and cheese borak and 20 weeks for sheesh tawoq treated with 6 kGy. Sensory evaluation showed no significant differences between irradiated and non-irradiated samples.

1. INTRODUCTION

Ready meals are meals that are cooked and stored in a frozen state (–18°C) until required for consumption. In recent years, the market for chilled ready meals that are cooked, stored under chilled conditions (0–3°C) and reheated prior to consumption has also shown substantial growth, largely as a result of

consumer perception that chilled foods are 'closer' to fresh than frozen foods [1–4].

Ready meals have a very limited shelf life at refrigeration temperatures and therefore there are concerns about the microbiological safety [5], sensory quality [6] and nutritive value [7]. Various methods, such as the modified atmosphere packaging, have been used to help preserve the sensory quality and to extend the shelf life of cook–chill foods [8]. However, many of these methods have not been developed to inactivate potential pathogens [9].

An alternative approach could be the use of medium dose irradiation to improve the microbiological safety and shelf life of chilled ready meals [1]. Irradiation treatment of ready-to-eat food can improve the shelf life and microbiological safety by destroying spoilage and pathogenic microorganisms [10–12].

Syrian consumers have recently started using ready meals which are prepared and marketed by local supermarkets. The Syrian food industry is traditionally dominated by kubba, borak and sheesh tawoq. Indeed, these meals are consumed not only in the Syrian Arab Republic, but also in neighbouring countries.

The objectives of this research were to investigate the use of gamma irradiation in order to improve the microbiological quality of certain kinds of Syrian precooked prepared meals (kubba, borak, cheese borak and sheesh tawoq) stored at low temperature (1–4°C), and to evaluate the changes in product quality and performance of irradiated products during storage.

2. MATERIALS AND METHODS

The experiments were conducted over four years on kubba, borak, cheese borak and sheesh tawoq. Each year one of these products was evaluated.

2.1. Preparation and formulation of kubba, borak, cheese borak and sheesh tawoq

The kubba, borak, cheese borak and sheesh tawoq were prepared by a local caterer. No changes were made to the way in which these meals were usually prepared in this industry.

Kubba has two parts, the outer layer of which consists of ground preboiled wheat (bulgar) mixed with minced beef (70% bulgar and 30% beef) and spices (allspice, black pepper, white pepper, onion and salt). The outer layer is stuffed with precooked lamb, onion, fat, pistachio and spices (allspice,

black pepper, white pepper, nutmeg, cumin and salt). After preparing, kubba is fried in sunflower oil for 2–3 min.

Borak has two parts; the outer part is a form of dough made of wheat flour (1 kg) mixed with 3 eggs, oil (200 mL), salt and water (300 mL). The dough is stuffed with a mixture of precooked lamb (1 kg), onion (200 g), fat (100 g), pistachio (200 g) and spices (black pepper, white pepper and salt).

Cheese borak has two parts; the outer part is a kind of dough and made of wheat flour (1 kg) mixed with 3 eggs, oil (200 mL), salt and water (300 mL). The slices of dough are stuffed with a mixture of uncooked baladi cheese.

After preparation, eight pieces of kubba, borak or cheese borak were placed on polystyrene trays and covered with lids made of polyethylene film. Each tray of borak was considered as a replicate.

Sheesh tawoq was prepared from boneless chicken breast, which was chopped into pieces of between 5 and 7 g each. To each kilogram of boneless chicken were added the following ingredients: 5 g ground garlic, 5 g coriander, 5 g white pepper, 5 g salt, 5 g cardamom, 5 g paprika, 10 g mustard, 5 g tomato paste, 10 g vinegar, 10 g olive oil, 30 g sunflower oil and 5 g lemon.

After preparation, 300 g of sheesh tawoq were placed on polystyrene trays and covered with lids made of polyethylene film. Each tray of sheesh tawoq was considered a replicate.

2.2. Treatments and analysis performed during storage

Samples from packed meals were exposed to gamma radiation at doses of 2, 4 and 6 kGy in a ^{60}Co package irradiator (dose rate 730 Gy/h). The irradiation was performed at room temperature (10–20°C). The absorbed dose was determined using an alcohol chlorobenzene dosimeter [13]. For each treatment, 20 trays of kubba, borak, cheese borak and sheesh tawoq were allocated and all were stored at refrigerated temperatures (1–4°C). Microbiological and chemical analyses were performed on controls and treated samples immediately after processing, and weekly throughout the storage period, which lasted 3 weeks for kubba, 6 weeks for borak and cheese borak and 20 weeks for sheesh tawoq. Sensory evaluation and proximate analysis were done within two days of irradiation.

2.3. Microbiological evaluation

Three replicates from each treatment were aseptically opened, and 10 g of the whole tested product was serially diluted according to standard methods [14]. The media used for the microbiological study were nutrient agar, for the total bacteria counts (TBC) (Oxoid, CM 325, United Kingdom) (48 h

incubation at 30°C). A cut-off value of 10^7 CFU/g for TBC [15] was used for the unacceptable samples and no further analyses were carried out when those indicator values were exceeded. Total coliforms were determined on violet red bile agar (Oxoid) at 37°C for 48 h. Yeasts were enumerated on dichloran rose–Bengal chloramphenicol agar (Merck) after incubation at 25°C for 5 d. In order to determine the survival curves before irradiation, the borak, cheese borak and sheesh tawoq were artificially inoculated by thoroughly mixing it with a pure culture of *Salmonella* spp. and *Escherichia coli*. The used *Salmonella* spp. and *E. coli* had been isolated in the laboratory from contaminated local food. The above mentioned strains were identified by biochemical tests. The initial numbers of artificial contamination were 1.2×10^7 and 2.0×10^6 CFU/g for *Salmonella* spp. and *E. coli*, respectively. The survival curve was estimated from irradiation doses of 0.20, 0.40, 0.60, 0.80 and 1.00 kGy. The survival level of *Salmonella* spp. was determined by plate counting on xylose lysine desoxycholate agar and the survival level of *E. coli* was determined by plate counting on eosin methylene blue agar after 2 d of incubation at 37°C.

2.4. Chemical evaluation

Approximately 150 g samples of the prepared products were blended for 15 s in a laboratory blender and subjected to chemical analysis. Each sample was homogenized and analysed in triplicate to determine moisture and ash (drying for 6 h at 105°C and ashing for 4 h at 550°C), fat (as extractable component in Soxhlet apparatus) and protein (as Kjeldahl nitrogen) contents using standard methods [16]. The pH values of the solutions of kubba, borak and cheese borak were determined using an HI 8521 pH meter (Hanna Instruments, United States of America).

2.5. Total acidity

The total acidity was obtained by a direct titration with 0.1N NaOH and phenolphthalein as an indicator [17]. Samples (10 g) of the meals were magnetically stirred in a total volume of 100 mL distilled water for 30 min and filtered. A 10 mL aliquot of the filtrate was titrated with 0.1N NaOH using 3 drops of a phenolphthalein indicator. The total acidity was calculated on the basis of 1 mL 0.1N NaOH = 0.0090 g lactic acid.

2.6. Total volatile basic nitrogen (VBN)

Samples (10 g) of the meals studied were mixed with 100 mL distilled water and washed into a distillation flask with 100 mL distilled water, followed

by 2 g of magnesium oxide and an antifoaming agent. The mixture was distilled using the micro-Kjeldahl distillation apparatus. Distillate was collected for 25 min into 25 mL of 4% boric acid and 5 drops of Tashero indicator added. The solution was titrated by 0.1N HCl to calculate the total VBN in the sample in terms of milligrams of VBN per kilogram of kubba, borak, cheese borak and sheesh tawoq (ppm) [18].

2.7. Lipid oxidation

Lipid peroxidation in terms of grams of iodine per 100 grams of fat of kubba, borak and cheese borak was determined by the modified method of Buege and Aust [19]. A fat sample of 1 g was placed in a 250 mL test flask and homogenized with a 20 mL solution of acetic acid (50% acetic acid, 50% chloroform). The mixture was vortexed, incubated in a hot water bath at 50°C for 30 min and the samples filtered. The filtrate was collected into 0.5 mL of potassium iodide (50%) and kept in a dark place for 2 min. Distilled water (100 mL) and 3 drops of starch 1% as an indicator were added, then the mixture was titrated by sodium thiosulphate pentahydrate (0.01N), added drop-wise until the end point.

2.8. Sensory evaluation

The sensorial criteria, especially the taste, odour, colour and texture of the irradiated and non-irradiated kubba, borak, cheese borak and sheesh tawoq were evaluated within two days of irradiation. In an equipped room, each panellist received four coded sample pieces (one non-irradiated and three irradiated samples, one at each dose). All the meals were tasted by 25 persons. Before testing, all samples of the meals were cooked. Kubba, borak and cheese borak were fried in sunflower oil for 5 min. Each member independently evaluated the kubba, borak, cheese borak and sheesh tawoq for taste, odour, colour and texture on a 5 point hedonic scale (1 = extremely poor, 2 = poor, 3 = acceptable, 4 = good, 5 = excellent), according to Ref. [20].

2.9. Statistical analysis

The four treatments were distributed in a completely randomized design with three replicates. The data were subjected to the analysis of variance test (ANOVA) using the SUPERANOVA computer package (Abacus Concepts Inc., USA (1998)). A separation test on treatment means was conducted using Fisher's least significant differences (LSD) methods at the $p < 0.05$ significance level. The D_{10} values were calculated using the Cricket graph computer package.

3. RESULTS

3.1. Results of the effect of gamma irradiation on kubba

3.1.1. Kubba characteristics

The mean of kubba characteristics in this study were: fat (12.16 ± 0.10%), protein (9.88 ± 0.75%), ash (1.93 ± 0.06%) and moisture (52.73 ± 0.43%).

Results of the proximate analysis of kubba showed that the moisture, protein and fat contents decreased in the samples irradiated with higher doses (4 and 6 kGy) (Table l).

3.1.2. Microbiological quality of irradiated kubba

Data in Table 2 indicate that 2, 4 and 6 kGy doses significantly ($p > 0.05$) decreased the TBC of kubba compared with the controls. The reduction was more than 1 or 2 log cycles for 4 and 6 kGy, respectively. Control and samples treated with 2 kGy presented a microbial load of 10^7/g [15] after one week of storage, whereas the samples treated with 4 or 6 kGy had not reached the same number after 3 weeks, representing satisfactory microbiological quality.

Therefore, the microbiological shelf life of kubba samples treated with 4 or 6 kGy was significantly extended from one week (control) to more than three weeks.

TABLE 1. EFFECT OF IRRADIATION ON MOISTURE, PROTEIN, FAT AND ASH CONTENTS OF KUBBA (%)

Treatment	Moisture	Protein	Fat	Ash
0 kGy	52.73 ± 0.43b*	9.88 ± 0.75b	12.16 ± 0.10b	1.93 ± 0.06b
2 kGy	51.09 ± 1.52ab	9.60 ± 0.47ab	12.09 ± 0.67b	1.86 ± 0.02a
4 kGy	50.53 ± 0.26a	9.67 ± 0.59ab	10.18 ± 0.40a	1.9 ± 0.04b
6 kGy	50.44 ± 1.24a	8.75 ± 0.38a	9.32 ± 0.53a	1.96 ± 0.01b
LSD	1.91	1.06	0.89	0.07

* Values within a column followed by the same letters are not significantly different at $p < 0.05$ significance level.

TABLE 2. EFFECT OF IRRADIATION ON MICROBIAL LOAD (ln CFU/g) OF KUBBA STORED AT 1–4°C

Dose (kGy)	Storage period (weeks)			
	0	1	2	3
0	2.96 ± 0.04a*	R**	R	R
2	2.20 ± 0.18ab	9.60 ± 0.47ab	R	R
4	1.71 ± 0.30b	1.52 ± 0.45a	0.97 ± 0.851a	3.89 ± 0.01a
6	0.93 ± 0.81b	0.87 ± 0.81a	1.00 ± 0.00a	2.15 ± 0.21b

* Values within a column followed by the same letters are not significantly different at $p < 0.05$ significance level.
** R: rejected.

3.1.3. Chemical quality of irradiated kubba

Total acidity: Table 3 shows that immediately after treatment, all doses (2, 4 and 6 kGy) had no effect on total acidity. After one week of storage, the total acidity of the samples treated with 4 and 6 kGy was significantly ($p < 0.05$) lower. Throughout storage periods, the total acidity of both irradiated and non-irradiated samples increased. The increase was higher in the control than in the irradiated samples.

TABLE 3. EFFECT OF IRRADIATION ON TOTAL ACIDITY (% LACTIC ACID) OF KUBBA

Dose (kGy)	Storage period (weeks)			
	0	1	2	3
0	0.18 ± 0.01a*	0.317 ± 0.030a	R**	R
2	0.16 ± 0.01a	0.244 ± 0.024a	R	R
4	0.18 ± 0.00a	0.202 ± 0.031b	0.222 ± 0.054a	236 ± 0.052a
6	0.17 ± 0.02a	0.224 ± 0.009b	0.244 ± 0.036a	0.211 ± 0.005a
LSD	0.03	0.047	0.104	0.084

* Values within a column followed by the same letters are not significantly different at $p < 0.05$ significance level.
** R: rejected.

VBN: There is an effect that irradiation treatments and storage time have on the VBN values (Table 4). Immediately after treatment, the VBN values of kubba samples treated with 2 and 4 kGy were significantly ($p < 0.05$) higher than the controls, whereas after one week of storage, these values in samples irradiated with 2, 4 and 6 kGy were significantly lower.

Lipid oxidation: The effects of gamma irradiation on lipid oxidation of kubba were compared (Table 5). Immediately after treatment, the lipid oxidation values of samples were no different from the non-irradiated ones. After one week of storage, the 4 and 6 kGy samples presented a significant ($p < 0.05$) increase in lipid oxidation. During storage, the lipid oxidation values of irradiated samples also increased.

TABLE 4. EFFECT OF IRRADIATION ON VBN OF KUBBA (ppm)

Dose (kGy)	Storage period (weeks)			
	0	1	2	3
0	76.0 ± 7.0a*	119.0 ± 1.0a	R**	R
2	106.0 ± 5.0b	62.3 ± 1.5b	R	R
4	138.0 ± 7.0c	77.0 ± 8.0cb	111.3 ± 1.5a	123.0 ± 11.0a
6	75.7 ± 2.5a	72.3 ± 10.5bc	101.3 ± 7.5a	105.0 ± 5.0a
LSD	10.7	12.6	12.3	19.4

* Values within a column followed by the same letters are not significantly different at $p < 0.05$ significance level.
** R: rejected.

TABLE 5. EFFECT OF IRRADIATION ON LIPID PEROXIDE IN KUBBA (g iodine/100 g fat)

Dose (kGy)	Storage period (weeks)			
	0	1	2	3
0	0.035 ± 0.001a*	0.042 ± 0.006a	R**	R
2	0.038 ± 0.008a	0.043 ± 0.007a	R	R
4	0.045 ± 0.02a	0.055 ± 0.005b	0.064 ± 0.05a	0.065 ± 0.011a
6	0.038 ± 0.005a	0.060 ± 0.0002b	0.068 ± 0.02a	0.078 ± 0.023a
LSD	0.023	0.010	0.008	0.041

* Values within a column followed by the same letters are not significantly different at $p < 0.05$ significance level.
** R: rejected.

TABLE 6. EFFECT OF IRRADIATION ON TASTE, TEXTURE, COLOUR AND FLAVOUR OF KUBBA*

Dose (kGy)	Taste	Flavour	Colour	Texture
0	4.00 ± 0.95a**	4.26 ± 0.97aa	4.27 ± 1.19a	3.83 ± 1.27a
2	4.00 ± 0.85a	4.26 ± 0.75a	4.46 ± 0.82a	3.83 ± 1.03a
4	4.08 ± 0.90a	4.08 ± 0.79a	4.64 ± 0.51a	4.33 ± 0.99a
6	4.08 ± 0.79a	3.83 ± 1.19a	4.27 ± 0.91a	4.25 ± 0.75a
LSD	0.72	0.78	0.77	0.84

* Data represent a 5 point scale ranging from 1 (very bad) to 5 (very good).
** Values within a column followed by the same letters are not significantly different at $p < 0.05$ significance level.

3.1.4. Sensory quality of irradiated kubba

Table 6 indicates that taste, odour, colour and textural characteristics of kubba samples were not affected by gamma irradiation treatment.

3.2. Results of the effect of gamma irradiation on borak

3.2.1. Borak characteristics

The mean characteristics of borak in this study were: fat (5.25 ± 0.60%), protein (8.06 ± 0.53%), ash (2.88 ± 0.27%) and moisture (39.03 ± 2.70%). The water activity value for borak was 0.95 at 24°C and the pH was 6.03 ± 0.04.

Results of the proximate analysis of borak showed that although irradiation affects the moisture, protein and fat contents and pH, the increase was not significant.

3.2.2. Microbiological quality of irradiated borak

Results of the microbial assessment of borak before and after irradiation are presented in Table 8. It was found that before irradiation, the samples presented TBC, total coliform and yeast values in the order of 10^6, 10^4 and 10^3 CFU/g, respectively. Irradiation with 2, 4 and 6 kGy of gamma irradiation significantly ($p > 0.05$) reduced TBC, total coliform and yeast content. The reduction was about 2, 4 and 6 log cycles for 2, 4 and 6 kGy, respectively. A dose of 6 kGy reduced the microbial load (TBC, total coliform and yeast) below the

TABLE 7. EFFECT OF GAMMA IRRADIATION ON MOISTURE, PROTEIN, FAT AND ASH CONTENTS OF BORAK (%)

Dose (kGy)	Moisture	Protein	Fat	Ash	pH
0	39.03 ± 2.70	8.06 ± 0.53	5.25 ± 0.60	2.88 ± 0.27	6.03 ± 0.04
2	45.87 ± 7.72	11.48 ± 0.13	6.36 ± 0.65	2.89 ± 0.80	5.93 ± 0.01
4	41.11 ± 0.82	12.15 ± 0.12	6.38 ± 0.98	2.96 ± 0.08	5.97 ± 0.05
6	43.93 ± 2.94	11.46 ± 0.16	5.85 ± 0.51a*	2.90 ± 0.12	5.87 ± 0.8
LSD	8.23	4.96	1.33	0.30	0.061

* Values within a column followed by the same letters are not significantly different at $p < 0.05$ significance level.

TABLE 8. EFFECT OF IRRADIATION ON MICROBIOLOGICAL QUALITY OF BORAK STORED AT 1–4°C

Dose (kGy)	Storage period (weeks)					
	0	1	2	3	5	6
TBC (ln CFU/g)						
0	6.17 ± 0.10	R[a]	R	R	R	R
2	4.08 ± 0.08	6.72 ± 0.29	5.35 ± 0.17	R	R	R
4	2.05 ± 1.38	6.18 ± 0.42	5.35 ± 0.17	5.88 ± 0.27	R	R
6	<1		3.1 ± 0.66	1.93 ± 1.42	3.45 ± 0.45	6.61 ± 0.40
TBC (ln CFU/g)						
0	4.14 ± 0.05	6.50 ± 0.10	R	R	R	R
2	2.57 ± 0.58	6.18 ± 0 .42	R	R	R	R
4	<1	4.78 ± 0.006	5.62 ± 0.07	4.54 ± 0.22	R	R
6	<1	<1	3.34 ± 0.11	<1	2.74 ± 0.30	5.25 ± 0.7
TBC (ln CFU/g)						
0	3.32 ± 0.13	8.90 ± 0.25	R	R	R	R
2	<1		R	R	R	R
4	<1	4.25 ± 1.03	4.67 ± 0.08	4.70 ± 0.14	R	R
6	<1	3.15 ± 1.24	4.10 ± 0.36	3.89 ± 0.15	4.83 ± 0.40	5.97 ± 0.07

[a] R: rejected.

accepted limit. Therefore, it was concluded that 6 kGy should be used for future studies.

The radiation doses required to reduce the microbial loads by one log cycle (D_{10} value) in Borak were 0.46 and 0.51 kGy for *Salmonella* and *E. coli,* respectively.

3.2.3. Chemical quality of irradiated borak

Total acidity: Table 9 shows that, immediately after irradiation treatment, the samples did not present changes in the total acidity, indicating that very few hydrolysis reactions occurred owing to irradiation treatment. After one week of storage, samples treated with 4 and 6 kGy presented a significant ($p < 0.05$) low total acidity. However, these values, both irradiated and non-irradiated samples, increased during the storage period.

VBN: Irradiation treatment and storage affected the VBN values (Table 4). Immediately after treatment (4 and 6 kGy), the VBN was significantly ($p < 0.05$) lower than in the controls. However, after one week of storage, there were no significant ($p < 0.05$) differences between treatments. Throughout the storage periods, the VBN concentration in the controls and samples irradiated with 2 kGy decreased, whereas the VBN of borak irradiated with 6 kGy increased.

Lipid oxidation: Immediately after treatment and one week of storage, the lipid oxidation values of irradiated samples were no different than those of non-irradiated controls. However, these values decreased during storage. This parameter, being a measure of rancidity, which is important for both the sensory and the nutritional points of view, would have been expected to increase in fat-containing products after irradiation treatment, especially at the higher doses employed. The data for this are not shown.

3.2.4. Sensory quality of irradiated borak

Sensory data presented in Table 10 indicate that taste, odour, colour and textural characteristics of borak were initially unaffected by gamma irradiation treatment. Sensory evaluation scores were essentially the same for both irradiated and non-irradiated samples.

TABLE 9. EFFECT OF IRRADIATION ON TOTAL ACIDITY, pH VALUE AND VBN OF BORAK STORED AT 1–4°C

Dose (kGy)	Storage period (weeks)					
	0	1	2	3	5	6
Total acidity (% lactic acid)						
0	0.28 ± 0.056	57.88 ± 17.09	R[a]	R	R	R
2	0.34 ± 0.06	46.22 ± 10.03	R	R	R	R
4	0.25 ± 0.006	25.64 ± 2.60	39.55 ± 12.69	45.04 ± 4.24	R	R
6	0.26 ± 0.05	26.53 ± 0.81	44.47 ± 17.69	41.86 ± 9.52	26.3 ± 2.84	27.09 ± 8.87
LSD	0.091	18.83	34.89	16.7		
VBN (ppm)						
0	251.47 ± 56.82	126.84 ± 43.47	R	R	R	R
2	209.14 ± 43.14	142.23 ± 16.06	R	R	R	R
4	137.52 ± 3.4	173.15 ± 7.06	151.39 ± 0.39	234.46 ± 13.47	R	R
6	106.77 ± 9.16	146.63 ± 16.43	239.30 ± 28.35	238.52 ± 11.21	156.19 ± 7.92	150.66 ± 8.09
LSD	67.79	46.76	45.72	28.03		
Liquid peroxide (g iodine/100g fat)						
0	0.15 ± 0.02	0.07 ± 0.01	R	R	R	R
2	0.16 ± 0.03	0.08 ± 0.06	R	R	R	R
4	0.13 ± 0.03	0.13 ± 0.08	0.052 ± 0.02	0.17 ± 0.07	R	R
6	0.16 ± 0.02	0.11 ± 0.04	0.083 ± 0.006	0.18 ± 0.05	0.41 ± 0.07	0.0076 ± 0.00365
LSD	0.05	0.1	0.026	0.075		

[a] R: rejected.

TABLE 10. EFFECT OF IRRADIATION ON TASTE, TEXTURE, COLOUR AND FLAVOUR OF BORAK[a]

Dose (kGy)	Texture	Flavour	Colour	Taste
0	3.82 ± 1.02	3.58 ± 1.36	3.77 ± 1.11	3.50 ± 1.27
2	3.35 ± 1.16	3.50 ± 1.48	3.27 ± 1.15	3.23 ± 1.24
4	3.58 ± 1.07	3.77 ± 1.28	3.27 ± 1.43	3.35 ± 1.20
6	3.85 ± 0.9	3.58 ± 1.39	3.27 ± 1.43	3.46 ± 1.42
LSD	0.57	0.76	0.65	0.73

[a] Data represent a 5 point scale ranging from 1 (very bad) to 5 (very good).

TABLE 11. EFFECT OF IRRADIATION ON MOISTURE, PROTEIN, FAT AND ASH CONTENTS OF CHEESE BORAK (%)

Dose (kGy)	Moisture	Protein	Fat	Ash
0	44.50 ± 1.34	11.15 ± 0.78	8.62 ± 2.06	1.60 ± 0.26
2	43.50 ± 0.72	11.56 ± 0.12	8.77 ± 1.77	1.10 ± 0.21
4	45.10 ± 1.64	10.35 ± 0.34	9.18 ± 1.12	1.58 ± 0.23
6	45.19 ± 1.32	10.17 ± 0.70	9.35 ± 1.35	1.45 ± 0.10
LSD	2.36	1.81	3.05	0.39

3.3. Results of the effect of gamma irradiation on cheese borak

3.3.1. Cheese borak characteristics

The mean characteristics of cheese borak in this study were: fat (8.62 ± 2.06%), protein (11.15 ± 0.78%), ash (1.60 ± 0.26%) and moisture (44.50 ± 1.34%). The water activity value for cheese borak was 0.92 at 24°C and the pH was 6.37 ± 0.04. Results of the proximate analysis of cheese borak showed that irradiation doses had no effect on moisture, protein or fat contents (Table 11).

3.3.2. Microbiological quality of irradiated cheese borak

The results of the microbiological analysis of cheese borak before and after irradiation are given in Table 12. It shows that the non-irradiated, tested cheese borak recorded TBC, total coliform and yeast in the order of 10^7, 10^5

TABLE 12. EFFECT OF IRRADIATION ON MICROBIOLOGICAL QUALITY OF CHEESE BORAK STORED AT 1–4°C

Dose (kGy)	Storage period (weeks)						
	0	1	2	3	4	5	6
Total microbial load (ln CFU/g)							
0	7.16 ± 0.24	R[a]	R	R	R	R	R
2	4.38 ± 0.09	6.45 ± 0.39	R	R	R	R	R
4	3.46 ± 0.33	4.94 ± 0.82	R	R	R	R	R
6	<1	<1	<1	<1	<1	<1	<1
Total coliform load (ln CFU/g)							
0	5.06 ± 0.21	R	R	R	R	R	R
2	2.57 ± 0.58	4.19 ± 0.13	R	R	R	R	R
4	<1	3.82 ± 0.14	R	R	R	R	R
6	<1	<1	<1	<1	<1	<1	<1
Total yeast load (ln CFU/g)							
0	4.62 ± 0.16	R	R	R	R	R	
2	3.08 ± 0.04	5.96 ± 0.59	R	R	R	R	
4	2.75 ± 0.30	4.74 ± 0.57	R	R	R	R	
6	<1	<1	4.84 ± 0.36	5.55 ± 0.65	5.24 ± 1.17	6.55 ± 0.6	6.84 ± 0.07

[a] R: rejected.

and 10^4 CFU/g, respectively. Irradiation with 2, 4 and 6 kGy of gamma irradiation significantly ($p < 0.05$) reduced TBC, total coliform and yeast in the cheese borak compared with the non-irradiated control. Irradiation with 2, 4 and 6 kGy reduced the TBC by about 2, 4 and 6 log cycles, respectively.

The radiation doses required to reduce the microbial loads by one log cycle (D_{10} value) were 0.30 and 0.50 kGy for *Salmonella* spp. and *E. coli,* respectively.

3.3.3. Chemical quality of irradiated cheese borak

Total acidity and pH: The total acidity of cheese borak samples are presented in Table 13. Immediately after treatment (2 and 4 kGy), the total acidity was similar for control and irradiated samples. After one week of storage the irradiated samples presented a significantly ($p < 0.05$) lower activity. These changes are reflected by the pH values.

VBN: The measurements of VBN were performed at the beginning and at weekly intervals for up to 6 weeks of storage. Data are presented in Table 13. It can be seen that immediately after treatment, the values of VBN in samples irradiated with 4 and 6 kGy were significantly ($p < 0.05$) lower than the control samples. Throughout the storage, the VBN values increased. Indeed, after one week of storage, the value of VBN in cheese borak irradiated with 2, 4 and 6 kGy was significantly ($p < 0.05$) higher than the control samples.

3.3.4. Sensory quality of irradiated cheese borak

Sensory testing (Table 14) showed that the taste, odour, colour and textural characteristics of cheese borak were initially unaffected by gamma irradiation. Sensory evaluation scores given by panellists were basically the same for both irradiated and non-irradiated samples. A correlation between sensory evaluation and chemical parameters was observed in relation to irradiated cheese borak.

3.4. Results of the effect of gamma irradiation on sheesh tawoq

3.4.1. Sheesh tawoq characteristics

The mean characteristics of sheesh tawoq in this study were: fat (9.84 ± 0.65%), protein (23.82 ± 1.13%), ash (1.78 ± 0.20%) and moisture (74.11 ± 0.49%), and the pH was 4.98 ± 0.25. Results of the proximate analysis of sheesh tawoq showed that irradiation doses had no effect on the moisture, protein and fat contents (Table 15).

TABLE 13. EFFECT OF IRRADIATION ON TOTAL ACIDITY, pH VALUE AND VBN OF CHEESE BORAK STORED AT 1–4°C

Dose (kGy)	Storage period (weeks)						
	0	1	2	3	4	5	6
Total acidity (% lactic acid)							
0	0.12 ± 0.01	0.57 ± 0.19	R[a]	R	R	R	R
2	0.12 ± 0.02	0.24 ± 0.03	R	R	R	R	R
4	0.10 ± 0.02	0.25 ± 0.03	0.19 ± 0.03	R	R	R	R
6	0.09 ± 0.01	0.19 ± 0.04	0.15 ± 0.01	0.20 ± 0.03	0.24 ± 0.09	0.25 ± 0.02	0.29 ± 0.02
LSD	0.03	0.19	0.03	16.7			
pH							
0	6.37 ± 0.4	4.72 ± 0.22	R	R	R	R	R
2	6.43 ± 0.01	5.86 ± 0.23	R	R	R	R	R
4	6.37 ± 0.04	6.02 ± 0.14	5.75 ± 0.09	R	R	R	R
6	6.39 ± 0.06	6.37 ± 0.04	6.34 ± 0.08	6.30 ± 0.02	6.30 ± 0.02	5.97 ± 0.07	5.58 ± 0.27
LSD	0.08	0.33	0.12				
VBN (ppm)							
0	32.28 ± 2.50	33.05 ± 9 9.84	R	R	R	R	
2	33.57 ± 0.39	58.95 ± 16.41	R	R	R	R	
4	28.40 ± 3.67	67.45 ± 4.64	41.84 ± 9.33	R	R	R	
6	23.29 ± 1.63	88.44 ± 20.94	72.57 ± 5.25	104.75 ± 0.03	107.48 ± 1.01	100.67 ± 7.82	151.91 ± 0.04

[a] R: rejected.

TABLE 14. EFFECT OF IRRADIATION ON TASTE, TEXTURE, COLOUR AND FLAVOUR OF CHEESE BORAK[a]

Dose (kGy)	Taste	Texture	Color	Taste
0	3.67 ± 1.13	3.79 ± 1.11	4.44 ± 0.92	3.72 ± 1.13
2	3.78 ± 1.00	3.83 ± 0.86	4.33 ± 0.84	3.78 ± 0.73
4	3.50 ± 1.15	3.56 ± 1.20	4.17 ± 1.04	3.28 ± 1.27
6	3.67 ± 0.91	3.33 ± 1.09	4.44 ± 0.71	3.28 ± 1.30
LSD	0.74	0.71	0.59	0.76

[a] Data represent a 5 point scale ranging from 1 (very bad) to 5 (very good).

TABLE 15. EFFECT OF IRRADIATION ON MOISTURE, PROTEIN, FAT AND ASH CONTENTS OF SHEESH TAWOQ (%)

Dose (kGy)	Moisture	Protein	Fat	Ash	pH
0	74.11 ± 0.49	23.82 ± 1.13	9.84 ± 0.65	1.78 ± 0.2	4.98 ± 0.25
2	73.44 ± 1.11	23.16 ± 0.45	8.55 ± 1.69	2.02 ± 0.13	5.22 ± 0.05
4	71.12 ± 1.47	25.10 ± 0.06	8.80 ± 0.17	1.47 ± 0.44	5.11 ± 0.37
6	73.93 ± 0.48	26.51 ± 0.13	11.15 ± 0.97	1.82 ± 0.21	4.81 ± 0.10
LSD	1.85	1.15	1.88	0.51	0.43

3.4.2. Microbiological quality of irradiated sheesh tawoq

The results of the microbiological analysis of sheesh tawoq before and after irradiation are given in Table 16. It shows that the non-irradiated sample recorded TBC, total coliform and yeast values of 10^4, 10^2 and 10^2 CFU/g, respectively. Irradiation with 2, 4 and 6 kGy of gamma irradiation significantly ($p < 0.05$) reduced the microbial load in the sheesh tawoq compared with the control samples.

The radiation doses required to reduce the microbial loads by one log cycle (D_{10}) in sheesh tawoq were 0.44 and 0.39 kGy for *Salmonella* spp. and *E. coli,* respectively.

TABLE 16. EFFECT OF IRRADIATION ON MICROBIOLOGICAL QUALITY OF SHEESH TAWOQ STORED AT 1–4°C

Dose (kGy)	Storage period (weeks)					
	0	4	8	12	16	20
0	4.76 ± 0.03	4.76 ± 0.03	4.76 ± 0.03	R[a]	R	R
2	3.88 ± 0.03	3.49 ± 0.08	3.45 ± 0.49	R	R	R
4	2.21 ± 0.18	2.91 ± 0.07	2.73 ± 0.03	2.62 ± 0.25	3.80 ± 0.02	2.68 ± 0.10
6	<1	<1	<1	<1	<1	<1
Total coliform load (ln CFU/g)						
0	2.33 ± 0.02	2.66 ± 0.04	3.49 ± 0.17	R	R	R
2	<1	<1	<1	R	R	R
4	<1	<1	<1	<1	<1	<1
6	<1	<1	<1	<1	<1	<1
Total yeast load (ln CFU/g)						
0	2.71 ± 0.05	5.74 ± 0.03	6.60 ± 0.03	R	R	R
2	2.00 ± 0.00	3.69 ± 0.08	5.52 ± 0.06	R	R	R
4	<1	2.58 ± 0.03	3.91 ± 0.02	4.28 ± 0.16	4.42 ± 0.23	4.57 ± 0.12
6	<1	<1	<1	<1	<1	<1

[a] R: rejected.

3.4.3. Chemical quality of irradiated sheesh tawoq

Total acidity and pH: The total acidity values are presented in Table 17. Immediately after treatment (2 and 4 kGy) there was no effect on this parameter, but during storage, the total acidity of non-irradiated samples increased. After 12 and 16 weeks of storage, 2, 4 and 6 kGy doses of gamma irradiation significantly ($p < 0.05$) decreased the total acidity.

The pH of sheesh tawoq was 4.98. Table 17 shows that the irradiation treatment had no effect on pH.

VBN: The measurements of VBN were performed at the beginning and at monthly intervals for up to 5 months of storage. Data presented in Table 17 indicate that immediately after treatment, the values of VBN of sheesh tawoq irradiated with 6 kGy doses of gamma irradiation were significantly ($p < 0.05$) higher than those of the control. After 4, 8, 12, 16 and 20 weeks of storage, the values of VBN of sheesh tawoq irradiated with 2, 4 and 6 kGy doses were significantly ($p < 0.05$) lower than those of the control.

3.4.4. Sensory quality of irradiated sheesh tawoq

Sensory evaluation (Table 18) showed that the taste, odour, colour and textural characteristics of sheesh tawoq were initially unaffected by gamma irradiation. Sensory evaluation scores were basically the same for both irradiated and non-irradiated samples.

4. DISCUSSION

Bognar [21] carried out a detailed study of the nutritive content of a variety of commercially prepared chilled, frozen, sterilized and fresh ready meals packaged into multiportion trays and it was found that on the day after preparation, there were no significant differences in the protein, fat and carbohydrate contents of the meals. Results of the proximate analysis showed that in the current studies, the irradiation doses applied had no effect on moisture, protein and fat of borak, cheese borak and sheesh tawoq.

A dose of 2 kGy was not effective in extending the shelf life of kubba. A dose of 2 kGy was, however, beneficial in improving the microbial quality of chilled meat products, including roast beef and ground beef patties [1, 23–26].

However, other authors have found that doses of 4 and 6 kGy reduced the microbial load and improved the shelf life of kubba, borak, cheese borak and sheesh tawoq, which agrees with the findings reported by Desmonts et al.

TABLE 17. EFFECT OF IRRADIATION ON TOTAL ACIDITY, pH VALUE AND VBN OF CHEESE SHEESH TAWOQ AT 1–4°C

Dose (kGy)	Storage period (weeks)					
	0	4	8	12	16	20
Total acidity (% lactic acid)						
0	2.9 ± 0.21	4.18 ± 10.56	3.93 ± 0.17	R[a]	R	R
2	2.88 ± 0.24	4.36 ± 2.7	3.17 ± 0.33	R	R	R
4	2.68 ± 0.25	3.97 ± 0.18	3.39 ± 0.33	3.65 ± 0.24	3.52 ± 0.5	2.97 ± 0.05
6	3.69 ± 0.16	3.3 ± 0.31	3.15 ± 0.04	3.53 ± 0.34	3.27 ± 0.1	3.02 ± 0.09
LSD	0.03	0.19	0.03	16.7		
pH						
0	4.98 ± 0.25	4.60 ± 0.25	4.95 ± 0.28	R	R	R
2	5.22 ± 0.05	4.86 ± 0.11	4.97 ± 0.21	R	R	R
4	5.11 ± 0.37	4.69 ± 0.03	4.86 ± 0.07	4.85 ± 0.07	4.93 ± 0.35	4.76 ± 0.13
6	4.81 ± 0.10	4.89 ± 0.22	4.79 ± 0.03	4.73 ± 0.39	4.80 ± 0.04	4.74 ± 0.1
LSD	0.43	0.33	0.34	0.38	0.38	0.75
VBN (ppm)						
0	543.52 ± 19.84	1049.72 ± 72.62	2029.1 ± 386.1	R	R	R
2	550.1 ± 17.1	780.62 ± 106.31	1450.48 ± 353.1	R	R	R
4	520.15 ± 29.75	671.92 ± 3.37	821.22 ± 80.22	804.6 ± 33.99	929.97 ± 95.04	694.86 ± 70.20
6	622.79 ± 11.93	724.22 ± 14.86	691.89 ± 33.34	746.01 ± 104.38	789.1 ± 43.49	761.82 ± 95.08
LSD	38.96	122.1	499.28	359.6	751.6	150.94

[a] R: rejected.

TABLE 18. EFFECT OF IRRADIATION ON TASTE, TEXTURE, COLOUR AND FLAVOUR OF SHEESH TAWOQ[a]

Dose (kGy)	Texture	Flavour	Colour	Taste
0	3.8 ± 0.89	3.83 ± 0.83	3.67 ± 1.1	3.50 ± 1.27
2	4.03 ± 0.96	4.03 ± 0.81	3.80 ± 1	3.23 ± 1.24
4	3.33 ± 0.92	3.47 ± 1.01	3.33 ± 0.71	3.35 ± 1.20
6	4.10 ± 1.9	3.60 ± 0.81	3.67 ± 0.71	3.46 ± 1.42
LSD	0.64	0.45	0.45	0.48

[a] Data represent a 5 point scale ranging from 1 (very bad) to 5 (very good).

[27]. In contrast, salmon patties irradiated at 6 kGy had a shelf life of 23 d at 4°C. Also, irradiation with 4 or 6 kGy and storage under refrigeration (5°C) increased the shelf life of corned beef [28] and chicken [29] from 10 d to 28 d.

Control samples of borak and cheese borak exceeded the limit of 10^7/g TBC [15] after one week of storage at 1–4°C. Samples irradiated at 6 kGy, however, remained acceptable after 6 weeks of storage at 1–4°C. These results are in agreement with the results of previous studies which indicated that 6 kGy was effective in extending the shelf life of precooked dishes [27], kebabs [30] and salmon patties [28].

Control samples of sheesh tawoq exceeded the limit of 10^7/g TBC [15] after 16 weeks of storage at 1–4°C. Samples irradiated at 2, 4 or 6 kGy, however, remained acceptable after 20 weeks of storage at 1–4°C.

Borak and cheese borak had high initial microbial numbers (10^6–10^7 CFU/g). A dose of 2 kGy had an effect in reducing the microbial counts of these meals and a slight effect in extending shelf life. Farkas et al. [23] and Paul et al. [24] found that irradiation of chilled meat products with a 2 kGy dose reduced the total viable cell counts and extended the microbiological shelf life of chilled meat. A dose of 2 kGy was beneficial in improving the microbial quality of the roast beef [1], meat as prepared food [25] and ground beef patties [26].

The radiation doses required to reduce the microbial loads by one log cycle (D_{10}) in borak, cheese borak and sheesh tawoq were estimated. Thayer [31] reported that the D_{10} values of *E. coli* and *Salmonella* spp. in ground beef at 0–4°C were 0.39 and 0.62 kGy, respectively. Mallett et al. [32] found that the D_{10} values of *E. coli* and *Salmonella* spp. in ground beef were 370 and 510 Gy, respectively, in oyster mantle fluid. The D_{10} values of *Salmonella* were 0.57, 0.42, 0.59, 0.46 and 0.49 Gy in ready prepared meals of roast beef, gravy, cauliflower, roast potatoes and mashed potatoes respectively [1]. Irradiation of

fresh chicken [33] and meat products [24] with a dose of 2.5 kGy seems to be effective in controlling *Salmonella* spp. contamination. However, there are indications that doses higher than 2.5 kGy may be required for complete elimination of *Salmonella* spp. in chicken [34].

The results of the effect of gamma irradiation on the total acidity of Syrian prepared meals (kubba, borak and sheesh tawoq) are in agreement with King et al. [22] who reported that no differences were found for the free fatty acids on day 0 and after 14 d of storage between the non-irradiated and irradiated beef, trout and pork at doses up to 3.5 kGy. Other authors [24, 35], however, indicated that the free fatty acid content in meat decreased after irradiation. The amount of lactic acid in irradiated nham was found to be lower than that in the non-irradiated samples after the same period of storage [36].

The values of lipid oxidation in kubba and borak indicate that these were within the acceptable limit.

The oxidative stability of fats in vacuum packaged products was reported by Taub et al. [37] who indicated that irradiation removed oxygen that remained in the head space or meat tissue of the vacuum packaged product, thus retarding autoxidation. On the other hand, Peter et al. [26] reported that no difference in lipid oxidation in ground beef patties was found within the first week of storage, regardless of packaging atmosphere. Paul et al. [24] also reported that meat treated with antioxidants prior to irradiation had lower values than untreated irradiated counterparts.

Luchsinger et al. [38] found that optimum packaging conditions can control rancidity changes in boneless chops, thereby enabling irradiation to be a useful intervention technology.

The VBN value, which is regarded as one of the standard chemical indices of freshness of meat, was assessed because the Syrian prepared meals (kubba, borak and sheesh tawoq) contained meat as the major component. After one week, irradiation resulted in a definite improvement in the keeping quality of the kubba and borak, as evidenced by their low VBN values compared with those of the control samples. The free radicals generated by irradiation can destroy antioxidants in muscle, reduce storability and increase total volatile compounds and off flavour production in meat [39, 40]. Dietary dl-α-tocopheryl acetate at >200 IU/kg decreased lipid oxidation and reduced the total volatility of raw turkey patties after 7 d of storage [41].

Many investigators have studied the effects of gamma irradiation on the sensory qualities of chilled ready meals. Most of them found that irradiation had no detrimental effects on the sensory quality of meals [1, 36, 38, 42].

Stevenson et al. [43] reported on a consumer trial carried out to assess the acceptability of a chilled irradiated (2 kGy) and non-irradiated ready meal. One hundred and seven consumers participated in this trial and found the

irradiated meal to be "moderately to very acceptable" and not significantly different from the non-irradiated meal. It therefore appears that untrained consumers may not be able to detect differences noted by trained panellists. Some studies reported sensory improvement of irradiated ready meals [27, 44, 45], whereas other studies revealed that the sensory qualities of ready meals are impaired when subjected to gamma irradiation. Sensory profiling techniques, using a trained panel of assessors, showed significant differences between the non-irradiated and irradiated chilled ready meals [1, 11]. Kilcast [46] 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. Lipid oxidation, which often occurs in uncured cooked irradiated products, also contributes to the flavour changes in irradiated meat products [26, 41].

5. CONCLUSION

From the results of this research it can be concluded that an irradiation dose of 6 kGy can inactivate all spoilage and pathogenic microorganisms in borak and cheese borak. The shelf life of locally prepared meals (kubba, borak and cheese borak) stored at 1–4°C was increased to more than 3 weeks, and to 6 weeks using doses of 6 kGy, as opposed to less than one week for controls for kubba and borak. Use of gamma irradiation has resulted in the production of locally prepared meals that are microbiologically safe without incurring any changes in their nutritional values. All samples of locally prepared meals were of acceptable sensory quality, even after the highest dose of 6 kGy was applied.

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USE OF IRRADIATION TO IMPROVE THE SAFETY AND QUALITY OF THAI PREPARED MEALS

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Abstract

Gamma irradiation was applied to extend the shelf life of selected Thai prepared meals, which comprised rice, meats and vegetables and which were kept under chilled conditions. For Thai spicy chicken basil rice (kao ka pao kai), cooked rice was prepared so as to obtain a harder texture and irradiated at 2 kGy. Three components (cooked chicken, sauce and blanched basil leaf) were separately packed and irradiated at 2 kGy for chicken and sauce and 0.1 kGy for basil leaves. The shelf life of irradiated spicy chicken (2 kGy) separately packed (>4 weeks) was much longer than the control sample (2 weeks), considering sensory quality. However, this dose was not enough to kill entirely the inoculated *Listeria monocytogenes* in spicy cooked chicken. Likewise, there is a need to preserve basil leaf, since it was microbiologically spoiled by the second week of storage. For stir-fried rice noodle with dried shrimp (pad Thai), a dose of 4 kGy was recommended because the product was free from *L. monocytogenes* and *Escherichia coli*, safe from microbial spoilage and had acceptable sensory quality. Irradiation at 4 kGy could extend the shelf life of chilled pad Thai to more than 4 weeks compared

with a normal chilled ready meal, which has a shelf life of 5–7 d. For steamed sticky rice, roasted chicken and papaya salad (kao neaw som tom), a dose of 3 kGy was enough to control *L. monocytogenes* and *E. coli* during chilled storage. The product irradiated at 3 kGy and upwards remained microbiologically safe after 8 weeks of chilled storage whereas the non-irradiated sample was spoiled after the second week. Panellists accepted irradiated steamed sticky rice and roasted chicken, which kept under chilled conditions for 8 weeks, but they rejected irradiated papaya salad owing to the soft texture and tainted taste.

1. INTRODUCTION

Many traditional Thai dishes are popular in Thai restaurants all over the world. Usually, they comprise herbs and spices with different types of meat and these are supplemented with rice. Thais prefer to eat aromatic rice or high amylose rice, but for those who come from the northern and northeastern parts of Thailand, 'sticky' rice is popular. The rice noodle is also a popular component in Thai cuisine. Rice noodle is cooked, added to soup containing various meats or stir-fried with different kinds of sauces. In this study, three of the famous Thai cuisines with various components were selected owing to their potential in commercial production.

However, apart from the traditional style of cooking at home or serving fresh in restaurants, there is increased consumption of prepared meals owing to the change in the lifestyle of people in both developed and developing countries. Such meals offer convenience and less preparation time. The meals are marketed in developed countries either chilled or frozen. Storage in the frozen state can extend the shelf life of prepared meals for several months, but this method is costly and needs to maintain the temperature at freezing to maintain the product's shelf life. Under chilled conditions, it is relatively more convenient, with only the need for a refrigerator for distribution and therefore easy for consumers to keep at home.

There is dynamic growth in the domestic market for chilled prepared meals because of the growth of food services in supermarkets and convenience stores. However, the shelf life for ready-to-eat chilled food, including chilled prepared meals, is quite short and sometimes insufficient to meet market requirements [1]. Furthermore, chilled foods, especially prepared and ready-to-eat meals, have been implicated in a number of serious foodborne disease outbreaks [2]. These were considered to be due to a new generation of refrigerated food [3], one which has partial processing to prevent normal spoilage bacteria but which also enhances the growth of pathogens.

Irradiation can provide the potential to improve the microbiological safety and extend the shelf life of chilled prepared meals. It can be used alone or combined with chilling. Using a combination of irradiation and chilling, frozen conditions can be replaced, resulting in energy savings and a lowering of operation costs. However, there is limited information about the application of irradiation on chilled prepared meals with respect to their shelf life quality and safety from foodborne pathogenic bacteria. Therefore, the study aims to investigate the possibility of using irradiation to extend shelf life and ensure the microbiological safety of three selected Thai chilled prepared meals: Thai spicy chicken and basil rice (kao ka pao ka), stir fried rice noodle with dried shrimp (pad Thai) and steamed sticky rice with roasted chicken and papaya salad (kao neaw som tom).

To ensure their safety from pathogens, these prepared meals were artificially contaminated with pathogenic bacteria including *Listeria monocytogenes, Escherichia coli* and *Salmonella typhimurium*. The radiation sensitivities of these bacteria were then determined, together with their survival after irradiation and during chilled storage, to suggest optimum irradiation doses. Apart from the microbial safety, the effects of irradiation on the products' physicochemical quality were evaluated and this is interesting because the selected meals comprise starch (steamed aromatic rice, sticky rice or rice noodle), protein (chicken or shrimp) and vegetables (basil leaf or papaya). The qualities were determined after irradiation and during storage under chilled conditions to assess their shelf lives.

2. REVIEWS

2.1. Microbiological contamination in chilled prepared meals

The US Food Safety and Food Inspection Service [1] provided information on refrigeration and food safety, including data on storage times of home refrigerated foods. The examples include cooked egg dishes, prepared salads, steaks or chops or roasts, soups and stews, and gravy and broth, whose shelf lives are around 3–4, 3–5, 3–5, 3–4 and 1–2 d, respectively. Besides the short shelf life, chilled foods are susceptible to foodborne disease outbreaks. Bryan [2] reported that numerous microbiological hazards and risks are associated with the preparation and storage of chilled foods in retail markets and food service establishments. Psychrotropic pathogenic bacteria such as *Clostridium botulinum* type E, *L. monocytogenes*, *Yersinia enterocolitica* and *Aeromonas hydrophila* continue to multiply during cold, but not frozen, storage. Microbiological spoilage occurs when the cold storage time exceeds

the shelf life of a product. Certain prepared foods are implicated repeatedly as vehicles in outbreaks of foodborne diseases. These include, in decreasing order of risk, roast beef, turkey, chicken, ham, other pork products, Mexican style foods, Chinese foods (usually rice), potato salad, rice, chicken salad, pizza and barbecue meat.

Chilled prepared meals were categorized by Corlett [3] as a new generation of refrigerated foods. Unlike traditional refrigerated foods, new generation products do not have a readily apparent preservation system. Furthermore, many of these products may have an extended shelf life and be packaged so as to inhibit 'normal' spoilage but which also enhances growth of pathogens. Other risk characteristics associated with new generation refrigerated products are inadequate temperature control, an inadequate distribution system and partial processing which prevents the normal warning of hazards by destruction of spoilage flora.

2.2. Radiation sensitivity of foodborne bacteria

Irradiation can damage and destroy most foodborne bacteria. *Salmonella* and *Listeria* proved to be more resistant to irradiation than *E. coli, Arcobacter*, *Campylobacter, Yersinia* and *Staphylococcus* [4–8].

2.2.1. L. monocytogenes

Among pathogenic bacteria, *L. monocytogenes* has the ability to grow over a wide range of environmental conditions such as heat and cold (from 1.1 to 50°C), low water activity and acid conditions [9] and, more importantly, it is resistant to irradiation. *L. monocytogenes* is significantly more resistant to irradiation in meats than in culture media [10–16]. Neither the fat content of meat [6] nor the source of raw meat (beef, chicken, lamb, pork, turkey (breast or leg)) had a significant effect on *D* values for irradiation [8, 15]. However, the effectiveness of a radiation dose in meat depended on cooking, the concentration of bacteria in the meat and the temperature during irradiation. When added to raw turkey nuggets, *L. monocytogenes* was more susceptible to radiation than when added to cooked turkey nuggets [17]. Radiation resistance was observed more at low temperatures [8, 13, 15]. With larger concentrations of *L. monocytogenes* in meat, higher doses were required to destroy the cells [13, 18].

Several researchers demonstrated that *L. monocytogenes* can grow in the cold and surviving and damaged cells may begin to multiply if the irradiated meat is stored under refrigeration [16, 19]. Food additives such as salt, nitrites and other compounds added to preserve meat have been found to enhance the

safety of irradiated foods as well as *L. monocytogenes* is more radiation resistant in uncured pork than in ham [20]. These techniques may act by increasing the effect of irradiation or by preventing the repair and growth of damaged and surviving cells. Among ready-to-eat meats, radiation resistance in terms of D_{10} values were 2.5 kGy for bologna, roast beef and smoked turkey and 3.0 kGy for frankfurters and ham. There was no difference between smoked turkey added with or without lactate [9]. However, when sodium diacetate and potassium lactate were added in beef bologna, radiation resistance was observed less [21] although this was not affected by dextrose concentration [22]. There was also a synergistic effect between pediocin and irradiation for inhibition of *L. monocytogenes* in frankfurters [23].

Some recommended doses to combat *L. monocytogenes* include:

- 3 kGy for elimination of 10^3 cells/g in air packed frozen chicken [14];
- 2.5 kGy to kill $10^{4.1}$ cells/g in ground beef [6];
- 2 kGy to destroy 10^4 cells/g in mechanically deboned chicken meat stored at 2–4°C [11].

Similar observations were made for vegetables. Niemira et al. [24] reported that the radiation sensitivities of the two strains, *L. monocytogenes* (ATCC 49594) and *Listeria innocua* (ATCC 51742), were similar when they were inoculated on endive. Type of vegetable (broccoli, corn, lima beans, peas, cabbage, tomato, mung bean sprouts) had a significant effect on the irradiation sensitivity of inoculated *L. monocytogenes* [25, 26]. Resistances increased with decreasing temperatures [25], reflected in the D_{10} values ranging from 0.51–0.61 kGy at –5°C to 0.77–0.92 kGy at –20°C.

It should be noted that various food additives and changes in processing parameters may affect the effectiveness of a radiation dose and that any surviving *L. monocytogenes* may grow to dangerous levels during storage at refrigeration temperatures. However, there has been very little published research on the effects of irradiation on prepared meals related to *L. monocytogenes* contamination.

Clardy et al. [27] found D_{10} values ranged from 0.71 to 0.81 kGy for *L. monocytogenes* in ready-to-eat ham and cheese sandwiches. Between the individual components of ready to eat sandwiches, Sommers and Boyd [28] reported D values of 0.27–0.37 kGy in tortilla and cheese interfaces, 0.33–0.41 kGy in cheese and turkey interfaces and 0.25–0.33 kGy in turkey and tortilla interfaces. Grant and Patterson [29] reported that radiation induced heat sensitization of *L. monocytogenes* and reheating kills contaminating pathogens in cooked, chilled roast beef and gravy.

2.2.2. *E. coli*

E. coli contaminates food through poor sanitation. Prepared meals are susceptible to this bacterium from many preparation methods. Radiation resistance of *E. coli* was different among strains when tested in media broth. Decreasing pH of the broth had relatively little effect on radiation resistance whereas induction of acid resistance to this type of bacteria consistently increased radiation resistance [30].

Many researchers studied radiation sensitivity of *E. coli*, especially an outbreak strain of *E. coli* 0157:H7, in many kinds of food. It was confirmed that not only in media but also in food did radiation sensitivity vary among strains. Rajkowski et al. [31] reported that D_{10} values of non-vegetable and vegetable isolated strains of *E. coli* 0157:H7 were 1.43 and 1.11 kGy, respectively. Niemira et al. [32] found that relatively subtle differences in lettuce types could significantly influence the radiation sensitivity of *E. coli* 0157:H7.

The nature of food contaminated with the pathogen also contributed to radiation sensitivity of the bacteria. Jo et al. [33] studied inactivation of *S. typhimurium*, *E. coli*, *Staphylococcus aureus* and *Listeria ivanovii* inoculated into prepared seafood products for manufacturing kimbab, steamed rice rolled in dried seaweed, by gamma irradiation. The D_{10} values of these organisms ranged from 0.23 to 0.62 kGy in imitation crab leg, 0.31–0.44 kGy in surimi gel and 0.27–0.44 kGy in dried seaweed. Chawla et al. [34] reported D_{10} values of *E. coli* in kwamegi, a traditional Korean semi-dried seafood, at 0.55 kGy.

Apart from strains and types of food, irradiation temperature also affected the inactivation of *E. coli* 0157:H7. The resistance in ground beef was significantly higher at sub-zero temperatures than above freezing, showing in D_{10} values for *E. coli* 0157:H7 in ground beef at 4 and –20°C of 0.39 and 0.98 kGy, respectively [35].

2.2.3. *S. typhimurium*

Salmonella spp. was among the important pathogens contaminating meats. This bacterium was also proved to be resistant to irradiation. Irradiation at a dose of 1 kGy eliminated *Salmonella* in raw meat samples sourced from butchers [36]. When raw beef mixed with and without sauce was artificially contaminated with the pathogens and irradiated up to 1.5 kGy, *D* values were higher at 0.37 and 0.55 kGy for *S. typhimurium* in raw meat with and without sauce than for *Y. enterocolitica* (0.043–0.080 and 0.10–0.21 kGy) and *Campylobacter jejuni* (0.08–0.11 and 0.14–0.16 kGy). A dose of 1 kGy is effective in reducing *Salmonella* by approximately 1.3–2.7 log cycles [37]. Irradiation at 1.25 and 2.50 kGy could reduce bacterial levels by 2.23 and 3.44 log cycles,

respectively, in mechanically deboned chicken meat. An insignificant change in the amount of *S. typhimurium* was found during storage for 9 d at 5°C [38]. The combined effect of gamma irradiation and heating on the destruction of *L. monocytogenes* and *S. typhimurium* was also studied in cooked, chilled roast beef and gravy [29].

2.2.4. Factors affecting radiation sensitivity

Niemira [39] studied the irradiation inactivation of four *Salmonella* serotypes in orange juices with various turbidities. It was found that the serotype was the more significant factor determining radiation resistance than turbidity or antioxidant power. Moreover, Niemira [39] reported that *Salmonella enteritidis* sensitivity to gamma irradiation was also not strongly influenced by the composition of formulated commercial orange juices in terms of the difference in pH, calcium concentration, juice composition and antioxidant power.

2.3. Application of irradiation for rice

Irradiation was introduced to paddy, brown and milled rice for the purpose of killing insects. Despite the fact that irradiation effectively controls insects, many studies reported the changes in rice quality either before or after cooking, such as colour, flavour, odour, cooking time, water absorption, viscosity, amylose content, oxidative rancidity, etc. Some studies recommended that the dose not exceed 1 kGy [40–42] while others argued that with doses above 1 kGy (1–5 kGy) milled rice was still accepted by consumers and the quality was not significantly changed [43–45]. However, there is limited information on cooked rice irradiation.

2.4. Application of irradiation for processed meat and fishery products

Numerous studies have been made on the effectiveness of irradiation in eliminating spoilage bacteria and pathogens in fresh and processed meat. Lacroix [46] determined if irradiation at a particular dose would be required to eliminate *Salmonella* completely on fresh poultry, combined with pretreatment. The results of sensory evaluation have shown significantly better odour and flavour for the irradiated chicken at 5 kGy than for the control. For cured meats, Shahidi et al. [47] reported that irradiation had no detrimental effects on the colour or flavour of either nitrite or nitrite-free cured samples. Fu et al. [7] showed there were no colour differences with respect to HunterLab values and visual evaluation scores between control and beefsteaks irradiated

at 1.5 kGy. However, an increase in redness due to irradiation has been stated for various raw and cooked pork products [4, 48, 49]. The improvement in ham colour by irradiation was also cited by Byun [50]. A dose of 5 kGy was observed to be as effective as the use of 200 ppm of sodium nitrite in providing and maintaining the desired colour of the product for 30 d, although it increased peroxidation of the product. However, the organoleptic quality of the irradiated ham without added sodium nitrite was acceptable. Sommers et al. [21] reported little effect on lipid oxidation or colour of the control bologna or bologna containing sodium diacetate and potassium lactate mixtures at either 1.5 or 3.0 kGy. A radiation dose of 3 kGy prevented the proliferation of *L. monocytogenes* and background microflora in bologna containing SDA-PL over 8 weeks of storage at 9°C. However, Sommers and Fan [22] found that a high dextrose concentration in combination with gamma irradiation increased lipid oxidation significantly. No growth of *L. monocytogenes* (inoculated at 10^5 CFU/g) was reported in ready-to-eat meats (frankfurters, ham, roast beef, bologna, smoked turkey) irradiated at 4.0 kGy stored at 4 and 10°C for 12 weeks. Survivors were observed for irradiated meats at 2.0 kGy stored at 10°C after the second week but it was not observed when stored at 4°C until the fifth week [9]. A combination treatment of vacuum–steam–vacuum and irradiation at 2.0 kGy did not cause significant changes in product structure, colour (redness) or lipid oxidation and resulted in an additive reduction of *L. innocua* on ham [51]. There was also a synergistic effect between pediocin and irradiation (1.2 or 2.3 kGy) for inhibition of *L .monocytogenes* in frankfurters. The treatments did not affect the sensory quality of the products [23].

For fresh seafood, Lacroix et al. [52] suggested a radiation dose of 2.5 kGy to eliminate pathogenic bacteria and increase the storage life of thawed shrimp for 1 month at cold temperatures (4°C) without any effects to quality. For processed fishery products, Adulpichit [53] reported that an irradiation dose of 4 kGy was more efficient in reducing the microbial load of semi-dried shrimps and shelf life could be extended to 7 weeks with respect to consumer acceptability and microbial safety compared with 10 d for the non-irradiated product. Four pathogens (*S. aureus, Bacillus cereus, S. typhimurium* and *E. coli*) inoculated in kwamegi, a traditional Korean semi-dried seafood, were eliminated by irradiation at 4 kGy. The product was safe during storage at –5 to 5°C for 4 weeks [34].

2.5. Application of irradiation for vegetables

Prakash et al. [54] studied the effects of 0.5 and 1.0 kGy gamma irradiation on the microbial and sensory characteristics of diced celery compared with conventional treatments such as acidification, blanching and

chlorination. Irradiation at 1.0 kGy could eliminate inoculated *L. monocytogenes* and *E. coli* and could extend the microbiological and sensory shelf life of diced celery compared with controls or other conventional treatments. Niemira et al. [25] studied the effect of irradiation on the quality of frozen vegetables. At –20°C radiation doses sufficient to achieve a 5 log cycle killing (3.9–4.6 kGy) of *L. monocytogenes* caused significant softening of peas and broccoli stems but not of corn or lima beans. Lower doses delivered at –5°C (2.5–3.1 kGy) did not cause significant changes in texture in any vegetable. Colour varied significantly among the dose–temperature combinations. Niemira et al. [24] found that a dose of 0.84 kGy, equivalent to a 99.99% reduction, suppressed *L. monocytogenes* throughout refrigerated storage. Doses up to 1.0 kGy had no significant effect on the colour and texture of endive leaf.

This was confirmed by Bari et al. [26], who reported the effective use of low dose irradiation to eliminate *L. monocytogenes* in fresh and freshly cut vegetables (broccoli, cabbage, tomatoes and mung bean sprouts). A dose of 1 kGy could reduce this pathogen by around 4.14–5.25 log CFU/g and the appearance, colour, texture, taste and overall acceptability did not undergo significant changes after 7 d of irradiation during storage at 4°C. Niemira et al. [32] also reported that doses of up to 0.5 kGy did not soften lettuce leaves. Doses ranged from 0.12 to 0.14 kGy and from 0.10 to 0.34 kGy and these could reduce the *E. coli* population by 90% when inoculated in lettuce leaf and homogenized leaf suspension, respectively. Rajkowski et al. [31] suggested that a dose of up to 2 kGy could extend the shelf life of broccoli sprouts, but doses >2 kGy would have an adverse effect on the broccoli seed and decrease the yield of broccoli sprouts. Similar results were presented by Bari et al. [55]. Treated at 1.5 and 2.0 kGy, *E. coli* was significantly reduced in mung bean and radish sprouts. Total vitamin C content was gradually reduced but the colour, firmness and overall visual quality of the tested sprouts was acceptable.

2.6. Application of irradiation for chilled prepared meals

Irradiation is known as an alternative means of extending the shelf life of chilled food in either raw or cooked form. Combined with another preservation method such as refrigeration, low dose irradiation can be applied to destroy contaminating organisms. Because of the low dose, changes in the sensory characteristics are small. Prepared meals ordinarily comprise two or more food components in one package and the use of irradiation for this type of product may be different and more complicated.

For salads, which comprise various kinds of fruits and vegetables, irradiation with doses to accomplish the intended purpose has resulted in

softening associated with changes in pectic substances. For pico de gallo, which is a cold Mexican style salad prepared by chopping and mixing fresh tomatoes, onions and jalapeno peppers, Howard et al. [56] reported that the colour, flavour, texture, odour and sensory attributes were not affected by radiation treatment (1 kGy). The treatment decreased populations of aerobic mesophilic, heterofermentative and total lactic microflora during storage. The L-ascorbic acid content declined 50% in response to gamma processing. Pectin solubility was also affected by radiation treatment.

For composite prepared meals, the IAEA [57] studied irradiation of meals which consisted of roast pork, gravy, mixed vegetables and boiled potatoes at doses of 1, 2 and 3 kGy. A dose of 2 kGy was found to be sufficient to extend the microbiological shelf life of the meals by at least 7 d. The microbiological results (total bacteria counts) 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. Reher et al. [58] studied the effectiveness of gamma irradiation (0.4, 0.8 and 3 kGy) on the microbial population, physical and chemical attributes and sensory stability of a refrigerated Salisbury steak, mashed potatoes and gravy meal packaged under a modified atmosphere. Irradiation appears to be an effective means of reducing pathogen counts in ready-to-eat meals without adverse effects on quality. The effectiveness of higher dose levels in eliminating *L. monocytogenes* and reducing yeast and mould counts in all meal components merits investigation. Clardy et al. [27] found that irradiation at 3.9 kGy could reduce *L. monocytogenes* contaminated in frozen ham and cheese sandwiches by 5 log units and it decreased further during storage at 4°C. Panellists could distinguish irradiated and non-irradiated products but the comments on whether irradiation adversely affected sandwich quality were divided. Lamb et al. [59] added that during refrigerated storage there was no growth of *S. aureus* in contaminated sandwiches when irradiated at 5.9 kGy. However, Sommers and Boyd [28] commented that the capability of irradiation to reduce pathogen levels on the complex tortilla, cheese and luncheon sandwiches was limited by the higher radiation resistance of *L. monocytogenes* when inoculated onto the ready-to-eat turkey meat components. The growth of *S. typhimurium, E. coli, S. aureus and L. ivanovii* inoculated into prepared seafood products for manufacturing kimbab, steamed rice rolled in dried seaweed, was inhibited by irradiation. *L. ivanovii* was not detected after a 3 kGy treatment, but the other pathogens were undetected at 2 kGy [33].

3. MATERIALS AND METHODS

3.1. Preparation of irradiated Thai prepared meals

3.1.1. Spicy chicken basil rice (kao ka pao kai)

Thai aromatic milled rice (KDML 105) and skinless boneless chicken breast and other ingredients such as vegetable oil, fish sauce, chilli and basil were purchased from a local market. Milled rice was soaked at 55°C for 1 h, boiled for 15 min and then steamed for 10 min. Then, each 200 g of cooked rice was packed in a plastic bag (nylon laminated with LDPE). To prepare spicy chicken basil, the fat of skinless boneless chicken breast was trimmed and removed and the meat chopped. The ingredients (vegetable oil, sliced chilli, basil, water and fish sauce) were added to the chopped chicken. They were cooked with a core temperature of chicken remaining above 70°C for 15 min. Then the 120 g of chicken basil was filled in each plastic bag (nylon/LDPE).

In this study, the cooked rice was prepared by three different methods:

(1) Soaking (25°C) 3 h, boiling 15 min, steaming 15 min (T1);
(2) Soaking (25°C) 3 h, boiling 13 min, steaming 15 min (T2);
(3) Boiling 15 min, steaming 15 min (T3).

Spicy chicken basil samples were packed differently by:

(1) Packing spicy chicken basil (T1);
(2) Packing cooked chicken meat with spicy sauce separately with blanched basil leaf (T2);
(3) Packing cooked chicken separately with spicy sauce and blanched basil leaf (T3).

The basil leaves were blanched in hot water at 98°C for 20 s [54]. After blanching, the basil leaves were rinsed with cold water and then packed in a nylon 15/DL/CPP 100 μm bag. The samples were irradiated at the Office of Atomic Energy for Peace, Bangkok, using a ^{60}Co Gammacell 220 facility (Nordion International Inc., Canada) at a dose rate of 0.086 kGy/min. The doses were varied to 1, 2, 3 and 4 kGy for preliminary study to find out the optimum dose for cooked rice and spicy chicken basil samples. The blanched basil leaves were irradiated at 0.1 and 0.2 kGy while the cooked rice and chicken and spicy sauce were irradiated at selected doses. Then, irradiated and non-irradiated samples were stored in a refrigerator (5 ± 1°C) for 4 weeks. Secondly, the cooked rice and spicy chicken basil were irradiated at 2 kGy. The

cooked rice was prepared by using T3 treatment. All irradiated samples were kept in a refrigerator (5 ± 1°C) for 4 weeks.

3.1.2. Stir-fried rice noodle with dried shrimp (pad Thai)

Dried rice noodles and dried shrimps were purchased from a local market. Spicy sauce (pad Thai sauce) was sourced from Pan Tai Norasingh Industry Co. To prepare pad Thai, 400 g of dried rice noodles were soaked in water for 10 min, then cooked in boiling water for 5 min. It was immediately cooled with cold water for a few seconds and then drained for 2 min. A 30 g sample of dried shrimps was blanched for 30 s in boiling water and 85 g of spicy sauce was heated to 85ºC using a hot plate. Hot sauce was added, mixed with the dried shrimp and then added with 5 g of vegetable oil. The cooked rice noodles and spicy sauce containing dried shrimp were separately packed in plastic bags (nylon/LDPE) and sealed. They were then irradiated at 0, 3 and 4 kGy and stored in a refrigerator under chilled conditions (5 ± 1°C) for 4 weeks.

3.1.3. Steamed sticky rice, roasted chicken and papaya salad (kao neaw som tom)

Sticky rice was soaked for 6 h, drained and placed over a thin white cloth. It was then steamed for 25 min. The chicken was mixed with the ingredients for 6 h and roasted in an oven at 100°C for 15 min. Papaya was washed, peeled and sliced. It was served with a dressing composed of fish sauce, lemon juice, garlic and sugar. Finally, 200 g of steamed sticky rice, roasted chicken and papaya salad were packed in nylon/LDPE bags.

3.2. Evaluation of radiation sensitivity of pathogens

3.2.1. Preparation of pure stock cultures and inoculums

A loopful of *L. monocytogenes* (4335) from the previous stock culture was transferred into 100 mL trypicase soy broth (TSB). For *E. coli* and *S. typhimurium*, 0.5 mL of lyophilized culture was added to each tube under aseptic conditions before being transferred into 100 mL of TSB. Both were put in an incubator at 37 $\pm$ 2°C for 24 h. The broth was then streaked into a petri dish with trypicase soy agar (TSA) and incubated for another 24 h at 37 $\pm$ 2°C. The single colonies were transformed into TSA slants and returned to the incubator for 24 h at 37 $\pm$ 2°C. The pure culture at this stage was kept in a refrigerator at 5°C and used as a stock culture of *L. monocytogenes, E. coli* and *S. typhimurium*. To prepare the inoculum, a loopful of a single colony from

each stock culture of *L. monocytogenes, E. coli* and *S. typhimurium* was transferred to 100 mL of TSB, shaken well to disperse the bacteria and incubated for 24 h at 37 ± 2°C. Standardization of each bacteria was done in TSB until the turbidity equalled MacFarland No. 0.5, which is equivalent to the bacteria concentration of 10^7 CFU/mL. The amount of bacteria in the inoculum was confirmed by the surface plate method.

3.2.2. Artificial contamination

For kao ka pao kai, cooked rice and spicy chicken without basil leaf were packed separately in OPP20/PE20/L-LDPE40 bags, sterilized at 121°C for 15 min and cooled to ambient temperatures. Sterilized cooked rice was inoculated with *L. monocytogenes* and *E. coli*, whereas spicy chicken basil was inoculated with *L. monocytogenes* and *S. typhimurium* at a concentration of 10^6 cells/g of sample. For pad Thai, *L. monocytogenes* and *E. coli* were separately inoculated into sterilized cooked rice noodles and dried shrimp in spicy sauce samples. The two pathogens were also inoculated into the sterilized steamed sticky rice, roasted chicken and papaya salad (kao neaw som tom).

3.2.3. Determination of D_{10} values

The cooked rice noodles and spicy sauce with dried shrimp (pad Thai) and steamed sticky rice, roasted chicken and papaya salad (kao neaw som tom), which were separately packed and inoculated with two selected pathogens, were irradiated at 1, 2, 3 and 4 kGy. After irradiation, survival of the two pathogens at each dose was determined. Total numbers of the two pathogens in contaminated samples were also determined in non-irradiated samples (the control sample). To calculate D_{10} radiation values, two methods were proposed. For the linear regression analysis method, the inactivation curves were plotted between log N/N_0 and D, where N is the quantity of surviving microorganism, N_0 is the initial number of organisms present and D is the dose absorbed by the product. The curves were fitted using least square regression through the data points in such a way that the non-linear parts were excluded. The D_{10} value was calculated from the negative inverse of the slope. For the total dose method, the D_{10} value is equal to the output from the amount of irradiation required to eliminate any survivors divided by the initial cell population (log CFU/g).

3.2.4. Evaluation of pathogen survival under chilled storage

The artificially contaminated cooked rice noodles and dried shrimp in spicy sauce (pad Thai), steamed sticky rice, roasted chicken and papaya salad

(kao neaw som tom) were irradiated at 3 and 4 kGy and kept in a refrigerator (5 ± 1°C), while the contaminated cooked rice and spicy chicken basil (kao ka pao kai) sample was irradiated at 3 kGy. After irradiation, samples were taken out weekly to evaluate the quantity of surviving pathogenic bacteria.

3.3. Shelf life evaluation

Optimum irradiation doses and shelf lives of three selected Thai meals were evaluated for changes of their physicochemical and microbiological properties after irradiation and during storage under chilled conditions (5 ± 1°C). For kao ka pao kai, the cooked rice was determined for microbial count, cooked rice hardness (back extrusion test), whiteness, pH and moisture content, whereas the spicy chicken basil was analysed for colour and pH. Both were tasted by panellists for sensory attributes using a scoring test (hedonic test). For pad Thai, cooked rice noodle was determined for moisture content, colour and noodle hardness (texture profile analysis by texture analyser) as well as pH and colour of spicy sauce and dried shrimp. Microbial quality was evaluated in terms of total plate counts and panellists were asked to evaluate the sensory quality (hedonic test). For kao neaw som tom, steamed sticky rice, roasted chicken and papaya salad were determined in terms of moisture content, colour and textural properties (texture profile analysis by texture analyser). Oxidative rancidity of the roasted chicken was determined by measurement of the thiobarbituric acid number (TBA).

4. RESULTS

4.1. Radiation sensitivity of pathogenic bacteria in chilled Thai prepared meals

4.1.1. Radiation sensitivity

Irradiation (D_{10}) values for *L. monocytogenes* and *E. coli* in cooked rice noodles and in spicy sauce with dried shrimp (pad Thai) under chilled conditions (5 ± 1°C) are shown in Table 1. The D_{10} value for *L. monocytogenes* was lower in cooked rice noodles. This may be because the spicy sauce had nutrients such as sugar, salt, tamarind juice and water to facilitate the growth of the bacteria whereas cooked rice noodles had only starch and water. The D_{10} value for *L. monocytogenes* in spicy sauce with dried shrimp was relatively higher compared to ready-to-eat meat such as frankfurters, ham, roast beef, bologna, smoked turkey (0.42–0.44 kGy at 4 and 10°C) [9] and chicken (0.77 at

TABLE 1. COMPARISON OF RADIATION D_{10} VALUES FOR *L. monocytogenes* AND *E. coli* IN COOKED RICE NOODLE AND SPICY SAUCE WITH DRIED SHRIMP (PAD THAI)

Type of pathogen	Type of substrate	D_{10} value	
		Linear regression analysis method (kGy)	Total dose method (kGy)
L. monocytogenes	Cooked rice noodle	0.27	0.27
	Spicy sauce with dried shrimp	0.69	0.67
E. coli	Cooked rice noodle	0.49	0.47
	Spicy sauce with dried shrimp	0.68	0.64

2–4°C) [11]. The D_{10} value for *E. coli* was also lower in cooked rice noodles. The nature of the substrate influenced the radiation sensitivity of pathogens [8, 11, 13]. The D_{10} value for *L. monocytogenes* in spicy sauce with dried shrimp was also relatively higher compared with meat such as minced chicken (0.35–0.39 kGy at 4°C), mechanically deboned chicken meat (0.28 and 0.44 kGy at 5 and –5°C) [12] and ground beef (0.24 and 0.31 kGy at 2–5°C and –17°C) [60]. Radiation can cause damage to microbial cells by destroying genetic materials and producing free radicals and other radiolytic products whose interaction with water can cause much more damage to cells [8]. It was determined that *E. coli* can adapt itself to grow and multiply in the two media of this study so well that it became more resistant than *E. coli* 0157:H7 in ground beef at 2–5°C. Compared with the D_{10} values obtained by the two methods, those from the linear regression analysis method were higher than those from the total dose method. It was suggested that the total dose method is appropriate to use when the low cell concentration of the microorganism has been applied because the skew in survival plots can introduce error. However, in this study, the initial cell concentration of 10^6 CFU/g was inoculated and therefore the linear regression analysis method, which resulted in a higher D_{10} value, might be capable of being applied in this case. The higher value obtained from the linear regression analysis method was confirmed by Andrews and Grodner [13].

Irradiation (D_{10}) values for *L. monocytogenes* and *E. coli* in steamed sticky rice, roasted chicken and papaya salad (kao neaw som tom) under chilled conditions (5 ± 1°C) are shown in Table 2. The D_{10} values of *L. monocytogenes* in steamed sticky rice were slightly higher than in cooked rice (0.27 kGy) as reported by Pungsawat [61]. There was a difference in the D_{10} values of this

TABLE 2. COMPARISON OF RADIATION D_{10} VALUES FOR *L. monocytogenes* AND *E. coli* IN STEAMED STICKY RICE, ROASTED CHICKEN AND PAPAYA SALAD (KAO NEAW SOM TOM)

Type of pathogen	Type of substrate	D_{10} value	
		Linear regression analysis method (kGy)	Total dose method (kGy)
L. monocytogenes	Sticky rice	0.33	0.37
	Roasted chicken	0.51	0.47
	Papaya salad	0.23	0.24
E. coli	Sticky rice	0.15	0.15
	Roasted chicken	0.52	0.48
	Papaya salad	0.44	0.42

pathogen determined by the two methods when inoculated in roasted chicken; they were slightly lower compared with those in ground beef and bologna [21]. The D_{10} values of *L. monocytogenes* in papaya slices were comparable to those of broccoli, mung bean sprouts, cabbage and tomato [55]. *E. coli* inoculated in steamed sticky rice seemed to be more susceptible to gamma irradiation than in cooked rice noodles [61], showing low D_{10} values. In roasted chicken, the D_{10} value of *E. coli* was comparable to that in minced turkey [62] but it was higher than that in minced chicken and pork [63]. The D_{10} values of *E. coli* in papaya salad were higher than those of *E. coli* 0157:H7 on the surface and in homogenized leaf suspension of lettuce [32].

4.1.2. Pathogen survival during storage of Thai prepared meals under chilled conditions

When contaminated kao ka pao kai was irradiated at 2 kGy, *E. coli* in cooked rice and *S. typhimurium* in spicy chicken basil were effectively reduced from a heavy contamination rate (10^8 and 10^4, respectively) to a safe level, and samples were free from these two pathogens until the end of storage (4 weeks) under chilled conditions (Table 3). Doses of 2 kGy also effectively killed all *L. monocytogenes* in the cooked rice, although it was not enough to decontaminate *L. monocytogenes* (CFU/g) in the spicy chicken basil. During chilled storage, the concentration decreased but then increased after 2 weeks. The inoculation rate of *L. monocytogenes* was high (2×10^7 CFU/g) compared with

TABLE 3. SURVIVAL OF PATHOGENS IN KAO KA PAO KAI AFTER IRRADIATION AT 2 kGy AND STORAGE UNDER CHILLED CONDITIONS

Samples	Initial concentration (CFU/g)	Concentration during chilled storage (CFU/g) Storage time				
		0 d	1 week	2 week	3 week	4 week
Control						
Cooked rice	<10	<10	<10	<10	<10	<10
	<10	<10	<10	<10	<10	<10
Spicy chicken	<10	<10	<10	<10	<10	<10
	<10	<10	<10	<10	<10	<10
L. monocytogenes						
Cooked rice	2.12×10^7	n.d.[a]	n.d.	n.d.	n.d.	n.d.
	2.25×10^7	n.d.	n.d.	n.d.	n.d.	n.d.
Spicy chicken	2.23×10^7	4.40×10^3	1.35×10^2	160	1.25×10^2	2.76×10^3
	2.05×10^7	4.85×10^3	1.40×10^2	130	1.30×10^2	2.58×10^3
S. typhimurium						
Spicy chicken	1.35×10^4	n.d.	n.d.	n.d.	n.d.	n.d.
	1.48×10^4	n.d.	n.d.	n.d.	n.d.	n.d.
E. coli						
Cooked rice	2.64×10^8	n.d.	n.d.	n.d.	n.d.	n.d.
	2.42×10^8	n.d.	n.d.	n.d.	n.d.	n.d.

[a] n.d.: not detected.

the microbiological quality of ready-to-eat foods at the point of sale whose threshold of 10^3 was unacceptable and potentially hazardous.

For pad Thai, no colony of contaminated *E. coli* was found in cooked rice noodles or spicy sauce with dried shrimp after irradiation at 3 or 4 kGy and storage for 4 weeks under chilled conditions (Table 4). It also happened for *L. monocytogenes* in spicy sauce with dried shrimp but injured *L. monocytogenes* in 3 kGy cooked rice noodles recovered in the second week of storage. *E. coli,* the Gram negative bacteria, was generally more susceptible to gamma irradiation than Gram positive bacteria because of the composition of the cell wall. Furthermore, irradiation has been reported to be an effective method for

TABLE 4. SURVIVAL OF PATHOGENS IN PAD THAI IRRADIATED AT 3 AND 4 kGy AND STORED UNDER CHILLED CONDITIONS

Samples	Initial concentration (CFU/mg)	Concentration during chilled storage (CFU/g) Storage time (weeks)				
		0	1	2	3	4
L. monocytogenes						
3 kGy						
Cooked rice noodle	1.59×10^7	<10	<10	80	285	190
Sauce and dried shrimp	1.03×10^6	<10	<10	<10	<10	<10
4 kGy						
Cooked rice noodle	1.59×10^7	<10	<10	<10	<10	<10
Sauce and dried shrimp	1.03×10^6	<10	<10	<10	<10	<10
E. coli						
3 kGy						
Cooked rice noodle	2.25 × 106	<10	<10	<10	<10	<10
Sauce and dried shrimp	1.79 × 106	<10	<10	<10	<10	<10
4 kGy						
Cooked rice noodle	2.25 × 106	<10	<10	<10	<10	<10
Sauce and dried shrimp	1.79 × 106	<10	<10	<10	<10	<10

the control of *E. coli* 0157:H7 [64]. It was proved in this study that an irradiation dose of 3 kGy was sufficient to eliminate contaminated *E. coli* in the two components of pad Thai stored under chilled conditions. For *L. monocytogenes*, this was confirmed by Foong et al. [9], who reported no survivors when 4 kGy was applied in ready-to-eat meat stored at 4 and 10°C for 12 weeks. When survival of both pathogens was considered, a dose of 4 kGy was recommended to ensure the safety of pad Thai during storage under chilled conditions.

After kao neaw som tom was irradiated at 3 and 4 kGy, there was no survival of *L. monocytogenes* during 4 weeks of storage (Table 5). Varabioff et al. [19] confirmed no recovery of *L. monocytogenes* in air packaged chicken irradiated at 2.5 kGy and stored at 4°C. There was also no survival of *E. coli* in this prepared meal when irradiated at 3 and 4 kGy and stored under chilled conditions.

TABLE 5. SURVIVAL OF PATHOGENS IN KAO NEAW SOM TOM IRRADIATED AT 3 AND 4 kGy AND STORED UNDER CHILLED CONDITIONS

Samples	Initial concentration (CFU/g)	Concentration during chilled storage (CFU/g) Storage time (weeks)				
		0	1	2	3	4
L. monocytogenes						
3 kGy						
Sticky rice	1.04×10^9	<10	<10	<10	<10	<10
Roasted chicken	3.82×10^8	<10	<10	<10	<10	<10
Papaya salad	1.63×10^8	<10	<10	<10	<10	<10
4 kGy						
Sticky rice	1.04×10^9	<10	<10	<10	<10	<10
Roasted chicken	3.82×10^8	<10	<10	<10	<10	<10
Papaya salad	1.63×10^8	<10	<10	<10	<10	<10
E.coli						
3 kGy						
Sticky rice	2.91×10^8	<10	<10	<10	<10	<10
Roasted chicken	1.85×10^8	<10	<10	<10	<10	<10
Papaya salad	3.93×10^8	<10	<10	<10	<10	<10
4 kGy						
Sticky rice	2.91×10^8	<10	<10	<10	<10	<10
Roasted chicken	1.85×10^8	<10	<10	<10	<10	<10
Papaya salad	3.93×10^8	<10	<10	<10	<10	<10

4.2. Shelf life extension of chicken basil rice (kao ka pao kai) by irradiation

4.2.1. Effect of irradiation on cooked rice and spicy chicken basil

For cooked rice (Table 6), the moisture content increased after irradiation, with significance observed at 4 kGy ($p < 0.05$). The cell structure of the components in rice may be ruptured by irradiation. Ionization can break chemical bonds resulting in loss of water holding capacity [65]. Cooked rice hardness decreased significantly ($p < 0.05$) at the lowest dose of 1 kGy. Reduction in cooked rice hardness after irradiation was confirmed [66, 67]. The

TABLE 6. EFFECT OF IRRADIATION ON QUALITY OF COOKED RICE AND SPICY CHICKEN BASIL

Irradiation dose (kGy)	Cooked rice					Spicy chicken basil	
	Moisture content (%)	Hardness	Whiteness (%)	Yellowness	pH	Redness	pH
0	71.23 ± 0.74a	16.30 ± 0.82d	63.43 ± 0.12e	3.68 ± 0.15a	6.17 ± 0.02	11.55 ± 0.73e	5.36 ± 0.03
1	71.81 ± 1.61ab	16.15 ± 0.29cd	62.70 ± 0.17d	4.42 ± 0.21b	6.18 ± 0.01	10.26 ± 0.16d	5.39 ± 0.02
2	72.02 ± 0.68ab	14.93 ± 0.18bc	61.83 ± 0.06c	4.48 ± 0.14b	6.20 ± 0.02	7.65 ± 0.15c	5.40 ± 0.01
3	73.02 ± 0.54ab	14.09 ± 1.18b	61.07 ± 0.06b	4.57 ± 0.13b	6.20 ± 0.01	6.30 ± 0.45b	5.41 ± 0.01
4	73.77 ± 1.79b	12.00 ± 0.23a	59.13 ± 0.06a	5.35 ± 0.11c	6.20 ± 0.01	5.28 ± 0.11a	5.41 ± 0.03

Note: Means for each characteristic followed by the same letter are not significantly different at $p < 0.05$ by LSD test.

colour of cooked rice changed from white to yellow after irradiation, with a significant difference noted at 1 kGy. This was also confirmed by the study of Wang et al. [43]. Gamma irradiation induces more reducing sugars and more maillard product formation. The pH did not change appreciably and there was no significant difference among the irradiation doses. The redness of chicken meat reduced (Table 6) significantly ($p < 0.05$) at 1 kGy due to the degradation of pigment in meat by the gamma irradiation process. The pH of chicken meat did not change much after irradiation. Panellists gave a lower score for cooked rice irradiated at higher dose (Fig. 1) owing to an unduly soft texture and to the slimy appearance of samples. As shown in Fig. 2, there was no significant

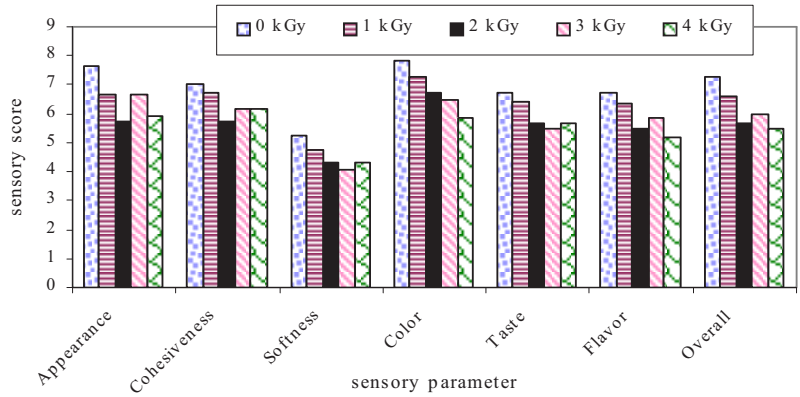

FIG. 1. Effect of irradiation on sensory quality of cooked rice.

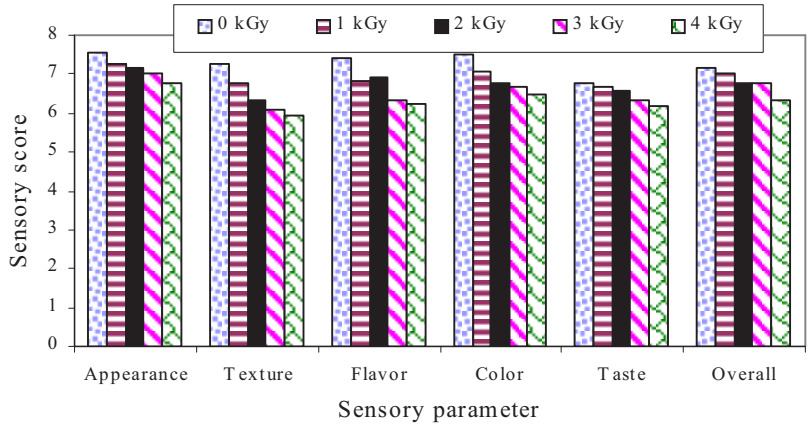

FIG. 2. Effect of irradiation on sensory quality of spicy chicken basil.

difference among overall acceptability scored for spicy chicken basil irradiated at 1–3 kGy, but it significantly reduced at 4 kGy ($p < 0.05$).

4.2.2. Shelf life of irradiated Thai spicy chicken basil rice during chilled storage

Doses of 1 and 2 kGy were selected based on the effects of irradiation on the quality of cooked rice and spicy chicken basil. To improve the cooked rice texture, the preparation method was changed (T1, T2 and T3). From Table 7, microbial populations reduced from 43.38 to 45.22% for irradiated cooked rice (T1–T3) and 45.84% for irradiated spicy chicken basil (T1). Irradiated cooked rice can be kept under chilled conditions for more than 1 month. For spicy chicken basil, the microbial population of T2 treatment, which was packed separately, was less probably due to the preservation effects of the spices in the

TABLE 7. MICROBIAL QUALITY OF NON-IRRADIATED AND IRRADIATED SPICY CHICKEN BASIL RICE DURING CHILLED STORAGE

Treatments (kGy)		Microbial population (CFU/g) Storage time (weeks)				
		0	1	2	3	4
Cooked rice						
T1	0	1.8256a	2.3482b	2.6713c	3.0324d	3.1849e
	1	1.0000a	1.1276b	1.1901b	1.5167c	1.8435d
T2	0	1.9002a	2.2876b	2.3567b	2.5862c	2.7734d
	1	1.0000a	1.0000a	1.2414b	1.5191c	1.6989d
T3	0	1.7919a	2.2563b	2.8065c	3.1374d	3.3006e
	1	1.0000a	1.1220ab	1.3383bc	1.4375c	1.6350d
Spicy chicken basil						
T1	0	2.9581a	4.4813b	5.5801c	6.8028d	
	2	1.6021a	2.1461b	2.5514c	2.9370d	3.3336e
T2	0	2.0792a	2.2553b	2.4771c	2.6590d	2.9943e
	2	<2.0000	<2.0000	<2.0000	<2.0000	2.0000
T3	0	2.1760a	3.3193b	3.7353c	7.0000d	
	2	<2.0000	<2.0000	<2.0000	<2.0000	2.0000

Note: Means for each characteristic followed by the same letter are not significantly different at $p < 0.05$ by LSD test.

sauce and reduced microbial contamination from basil leaves. There was a slight change of microbial growth in irradiated spicy chicken basil, especially when components were packed separately (T2 and T3). These could be kept for more than 4 weeks compared with 3 weeks for the control sample. In terms of sensory quality, T2 obtained the lowest score among three different cooking methods whereas T3 received more preference. This may be due to the unduly soft texture of the T2 sample. Therefore, T3 was the suitable cooking method with which to prepare irradiated cooked rice.

For a more practical process, cooked rice and spicy chicken basil should be irradiated at the same dose (2 kGy). With this condition, panellists accepted irradiated cooked rice after 4 weeks of storage compared with the control sample, which was rejected at 3 weeks (Table 8). For irradiated spicy chicken basil (T1), the sensory acceptability decreased during storage mainly due to the development of an off flavour, which was associated with the rapid increment in acetaldehyde and ethanol during storage [54] and the colour change of irradiated basil leaf. As recommended by Prakash et al. [54], the irradiation dose for vegetables should be 0.1–0.35 kGy. Hence, it was necessary to treat the

TABLE 8. SENSORY QUALITY OF COOKED RICE PREPARED WITH T3 METHOD AND TREATED WITH 2 kGy AND WITHOUT IRRADIATION DURING CHILLED STORAGE

Treatment (kGy)	Storage time (weeks)	Sensory attributes				
		Cohesiveness	Softness	Flavour/ aroma	Colour	Overall acceptability
0	0	7.0b	6.8c	6.0c	7.6b	6.8c
	1	7.0b	6.6c	5.8c	7.4ab	6.6bc
	2	6.6b	6.2bc	5.4ab	7.2ab	6.2bc
	3	5.0a	5.6b	5.2ab	7.0a	5.8b
	4	4.2a	4.2a	4.8a	7.0a	4.6a
2	0	6.6c	6.8c	5.4	7.4	6.6b
	1	6.2bc	6.6c	5.4	7.2	6.4b
	2	6.0abc	6.4bc	5.4	7.0	6.4b
	3	5.4ab	5.8ab	5.2	7.0	5.2a
	4	5.2a	5.4a	4.8	7.0	5.0a

Note: Means for each characteristic followed by the same letter are not significantly different at $p < 0.05$ by LSD test.

basil leaf with a lower irradiation dose and keep it separate from the chicken (T2 and T3). The basil leaf was irradiated at 0.1 kGy.

From Table 9, it can be seen that T2 (mixed sauce with chicken) treated with or without irradiation could be kept for more than 3 weeks. However, the effect of irradiation could be seen clearly in T3 (separate chicken and sauce) where shelf life of the irradiated sample (>4 weeks) was longer than the control sample (2 weeks).

TABLE 9. SENSORY QUALITY OF SPICY CHICKEN BASIL PACKED DIFFERENTLY AND TREATED WITH 2 kGy AND WITHOUT IRRADIATION DURING CHILLED STORAGE

Treatment (kGy)		Storage time (weeks)	Sensory attributes				
			Texture	Juiciness	Flavour/ aroma	Colour	Overall acceptability
T2	0	0	5.6	6.2c	6.0c	7.0c	6.4c
		1	5.4	5.8bc	5.8bc	6.6c	6.2bc
		2	5.2	5.2ab	5.2bc	6.4bc	5.8bc
		3	5.0	5.0ab	4.8ab	5.8b	5.4b
		4	4.8	4.8a	3.8a	5.4a	4.4a
	2	0	5.8b	6.2	6.0b	7.0b	6.4b
		1	5.6ab	6.0	5.8ab	6.8ab	6.0b
		2	5.4ab	5.8	5.6ab	6.6ab	5.8b
		3	5.0ab	5.4	5.4ab	6.4ab	5.8b
		4	4.8a	5.0	5.2a	6.2a	4.8a
T3	0	0	5.6b	6.4c	7.0c	6.6	6.6c
		1	5.4b	6.2bc	6.8c	6.4	6.4bc
		2	5.2b	5.6b	5.6b	6.4	5.8b
		3	4.0a	4.4a	3.8a	6.4	4.4a
	2	0	5.4b	6.4b	6.8b	6.6b	6.4b
		1	5.4b	6.2b	6.2ab	6.4b	6.2b
		2	5.0ab	5.8ab	5.8ab	6.2ab	6.0ab
		3	4.6ab	5.4ab	5.4a	5.6ab	5.8ab
		4	4.2a	5.0a	5.4a	5.2a	5.2a

Note: Means for each characteristic followed by the same letter are not significantly different at $p < 0.05$ by LSD test.

However, this result was from a sensory test that did not include basil leaves because it was microbiologically spoiled by the second week of storage.

4.3. Shelf life extension of stir-fried rice noodle with dried shrimp (pad Thai) by irradiation

4.3.1. Quality of cooked rice noodle

After irradiation at 3 and 4 kGy, the moisture content of irradiated cooked rice noodle increased. This was confirmed by Lu et al. [68], who found an increase in the moisture content of irradiated sweet potato, and Sabularse et al. [69], who reported that modification of the starch structure caused an increase in the water uptake during the cooking of rice. During storage under chilled conditions, the moisture content of all samples decreased significantly by around 2% (Table 10) owing to the diffusion of water vapour from food to storage environments. The lightness value of cooked rice noodles decreased after irradiation at 3 and 4 kGy. Likewise, an increase in yellowness was observed. This was confirmed by Koomsanit [70], who found the colour of cooked rice changed from white to yellow after exposure to radiation, even at 1 kGy. Maillard's reaction was cited to be a reason for the increase in yellowness of the rice products after irradiation [43]. However, during storage under chilled conditions, the lightness value of all samples tended to increase with time (Table 10) owing to more opacity of the rice noodles resulting from reassociation and crystal formation of the amylopectin fraction in gelatinized starch [71]. Whereas the yellowness decreased slightly with storage time for the non-irradiated cooked rice noodles, it did not change significantly ($p < 0.05$) in irradiated noodles. The retrogradation of starch in the cooked rice noodles during chilled storage made for opacity of the products and resulted in a decrease in yellowness. However, irradiation initially caused increased yellowness in noodle colour, so that the observed decrease was less evident. Reduced hardness of the cooked rice noodles was noticed after irradiation at 3 and 4 kGy; it was dose dependent. This might be due to a breakdown of starch molecules induced by gamma irradiation. Furthermore, this was observed together with an increase in the moisture content of cooked rice noodles after irradiation. The texture of irradiated cooked rice noodles was softer and their appearance slimy. When noodle samples were kept under chilled conditions, their hardness increased significantly ($p < 0.05$) with storage time (Table 10). However, owing to the breakdown of starch molecules after irradiation, lower hardness of the cooked rice noodles irradiated at 3 and 4 kGy was observed at the end of storage, compared with the non-irradiated sample. The harder

TABLE 10. QUALITIES OF NON-IRRADIATED AND IRRADIATED PAD THAI DURING CHILLED STORAGE

Components	Quality attributes	Storage time (weeks)	Irradiation dose (kGy)		
			0	3	4
Cooked rice noodle	Moisture content (%)	1	68.47 ± 0.28aa	69.10 ± 0.17a	69.59 ± 0.39a
		2	68.28 ± 0.40a	68.67 ± 0.29ab	68.84 ± 0.04b
		3	67.24 ± 0.08b	68.41 ± 0.04b	68.31 ±0.50b
		4	66.51 ± 0.34c	67.39 ± 0.51c	67.37 ± 0.12c
	Lightness	1	72.53 ± 0.08b	70.20 ± 0.09a	69.43 ± 0.08b
		2	72.59 ± 0.06b	70.52 ± 0.04b	69.38 ± 0.07b
		3	72.64 ± 0.05b	70.62 ± 0.03b	69.22 ± 0.09a
		4	72.80 ± 0.12a	70.82 ± 0.07c	69.78 ± 0.07c
	Yellowness	1	7.82 ± 0.07a	9.07 ± 0.17a	9.67 ± 0.20a
		2	7.72 ± 0.17ab	9.15 ± 0.19a	9.69 ± 0.05a
		3	7.57 ± 0.07b	9.21 ± 0.05a	9.82 ± 0.28a
		4	7.34 ± 0.10c	9.24 ± 0.33a	9.83 ± 0.29a
	Hardness	1	40.44 ± 0.14a	36.64 ± 2.01a	33.30 ± 0.58a
		2	44.36 ± 3.09b	39.88 ± 1.36b	37.72 ± 2.64b
		3	45.71 ± 0.58bc	41.94 ± 2.43bc	39.66 ± 1.13b
		4	48.31 ± 0.79c	44.78 ± 1.35c	40.63 ± 2.16b
Spicy sauce	Redness	1	4.10 ± 0.10a	3.36 ± 0.02a	3.19 ± 0.07a
		2	3.23 ± 0.06b	2.82 ± 0.02b	2.44 ± 0.07b
		3	2.86 ± 0.03c	2.29 ± 0.09c	2.37 ± 0.12b
		4	2.59 ± 0.01d	2.02 ± 0.09d	1.83 ± 0.01c
	pH	1	4.55 ± 0.01a	4.82 ± 0.01a	4.86 ± 0.01a
		2	5.26 ± 0.00b	5.33 ± 0.01b	5.41 ± 0.01b
		3	5.21 ± 0.01c	5.54 ± 0.01c	5.72 ± 0.01c
		4	5.44 ± 0.02d	5.69 ± 0.00d	5.76 ± 0.01d
Dried shrimp	Lightness	1	43.57 ± 0.05a	43.48 ± 0.06a	45.74 ± 0.09a
		2	40.26 ± 0.07b	41.44 ± 0.02b	44.05 ± 0.04b
		3	41.02 ± 0.05c	41.05 ± 0.05c	39.17 ± 0.02c
		4	41.25 ± 0.03d	41.55 ± 0.03d	41.41 ± 0.05d

TABLE 10. QUALITIES OF NON-IRRADIATED AND IRRADIATED PAD THAI DURING CHILLED STORAGE (cont.)

Components	Quality attributes	Storage time (weeks)	Irradiation dose (kGy)		
			0	3	4
	Redness	1	21.96 ± 0.07a	18.76 ± 0.09a	17.01 ± 0.05a
		2	18.27 ± 0.06b	17.63 ± 0.04b	16.92 ± 0.04b
		3	19.06 ± 0.06c	17.06 ± 0.05c	16.63 ± 0.04c
		4	18.45 ± 0.03d	15.26 ± 0.02d	15.95 ± 0.04d

Note: Means for each characteristic followed by the same letter in each sample during 4 weeks of storage are not significantly different at $p < 0.05$ by LSD test.

texture of starch based products which occurred during cold storage was explained by retrogradation [72].

4.3.2. Quality of spicy sauce

After irradiation, the lightness of spicy sauce decreased because of the reflection from vegetable oil floating on the sauce surface. The redness decreased with gamma dose. Gamma irradiation may cause the degradation of the colour pigments in spicy sauce, but there was no significant change in yellowness after irradiation. During chilled storage, the redness value gradually decreased in the non-irradiated sauce but decreased more in the irradiated sauce (Table 10). There was an increase in pH due to the radiolysis of water in the product after irradiation. The pH of the spicy sauce significantly increased ($p < 0.05$) during storage in both the non-irradiated and irradiated samples (Table 10). The increase in pH was evident, especially at high doses. The sauce comprised water, tamarind juice, sugar, salt, acetic acid and paprika oleoresin colour. The majority of the components, such as water, may change by radiolysis, induced by gamma irradiation [73]. The consequences of the radiolysis reaction are free radicals, which then react to other food molecules and become radiolytic products. The highly reactive entities formed can evaporate rapidly during irradiation treatment. Consequently, the concentration of solutes in spicy sauce was increased and resulted in a slightly salty taste. Thus, increase in pH during storage might be due to the sauce becoming more concentrated after irradiation and during storage.

4.3.3. Quality of dried shrimp

After irradiation, the lightness of the dried shrimp increased but the redness value decreased. This was confirmed by Adulpichit [53]. Irradiation may cause the degradation of the main pigment (astaxanthin) in dried shrimp. Astaxanthin comprises terpenoid, which provides the yellow, orange and red colour of the products and therefore oxidation of astaxanthin by gamma irradiation induced a paler colour of dried shrimp. The redness continually decreased during chilled storage (Table 10). The sauce and dried shrimp were packed in an abundance of oxygen and this may have been responsible for the oxidation of astaxanthin, resulting in discoloration of the dried shrimp.

4.3.4. Microbial quality of irradiated pad Thai

It was expected that irradiation would extend the shelf life from 5 to 7 d for normal chilled prepared meals to 4 weeks. Samples were not at risk of spoilage because the cooked rice noodles and spicy sauce with dried shrimp were packed separately. Likewise, the lower microbial load was in cooked rice noodles, which were boiled for 5 min, and in dried shrimp, which was blanched in hot water before packing, and also in spicy sauce, which had a low pH. However, under chilled storage, the microbial load grew in cooked rice noodles as observed by higher microbial counts in the irradiated and non-irradiated samples (1.3–6.2 ln CFU/g) at the end of storage compared to spicy sauce (<1–2.3 ln CFU/g) (Table 11). This may be because the spicy sauce had a low pH,

TABLE 11. TOTAL PLATE COUNTS OF NON-IRRADIATED AND IRRADIATED PAD THAI DURING CHILLED STORAGE

Components	Irradiation dose (kGy)	Population density (ln CFU/g)				
		Storage time (weeks)				
		0	1	2	3	4
Cooked rice noodle	0	1.81	2.26	3.95	5.10	6.15
	3	1.00	1.18	1.88	3.84	4.30
	4	<1.00	1.00	1.00	1.18	1.30
Spicy sauce with dried shrimp	0	1.00	1.40	1.74	2.10	2.29
	3	1.00	1.00	1.18	1.30	1.48
	4	<1.00	<1.00	<1.00	<1.00	<1.00

which can inhibit the growth of bacteria. With irradiation, fewer microbial counts were noticed in both components, especially at a high gamma dose. With respect to criteria for food spoilage at 7 ln CFU/g, pad Thai irradiated at 4 kGy had a shelf life much longer than 4 weeks.

4.3.5. Sensory quality of irradiated pad Thai

To serve in a sensory test, cooked rice noodle was mixed with the spicy sauce and dried shrimp. From Table 12, after irradiation, panellists preferred non-irradiated to irradiated pad Thai. Among the samples, pad Thai irradiated at 4 kGy had a lower overall acceptability score during the early period of storage (1–2 weeks). Panellists commented that irradiated pad Thai appeared slimy and the texture was too soft. Moreover, the flavour of a 4 kGy sample seemed to be more salty than non-irradiated and lower dose irradiated noodles. However, after 3 weeks of storage, overall acceptability of pad Thai irradiated at 4 kGy was better and comparable to other samples. This was consistent with an increase in hardness of the cooked rice noodle during storage as determined by objective methods.

TABLE 12. SENSORY QUALITY OF NON-IRRADIATED AND IRRADIATED PAD THAI DURING CHILLED STORAGE

Irradiation dose (kGy)	Storage time (weeks)	Sensory scores					
		Appearance	Texture	Colour	Taste	Flavour	Overall preference
0	1	7.2a	6.2a	6.7ab	6.4b	6.4a	6.8a
	2	7.0a	6.6a	6.3a	5.6a	5.9a	6.2ab
	3	7.5a	6.0a	7.2b	6.1ab	6.5a	6.2ab
	4	6.7a	6.0a	6.1a	5.4a	5.6a	5.7b
3	1	7.0b	6.4a	6.6b	6.6a	6.4b	6.5a
	2	6.1a	6.6a	5.4a	5.3a	5.3a	6.2a
	3	7.1b	7.2a	6.4b	6.0a	5.6ab	6.2a
	4	6.6ab	7.3a	6.0ab	5.6a	5.2a	6.7a
4	1	6.6ab	5.7a	6.6a	5.6a	5.4a	5.7ab
	2	5.7a	5.6a	5.8a	5.4a	5.7a	5.4a
	3	7.0b	6.9b	6.1a	6.1a	6.0a	6.4b
	4	6.5ab	7.2b	6.0a	6.0a	6.1a	6.4a

Note: Means for each characteristic followed by the same letter in each sample during 4 weeks of storage are not significantly different at $p < 0.05$ by LSD test.

4.4. Shelf life extension of steamed sticky rice, roasted chicken and papaya salad (kao neaw som tom) by irradiation

4.4.1. Quality of steamed sticky rice

From Table 13, the moisture content of non-irradiated steamed sticky rice did not change during chilled storage but increased slightly for irradiated sticky rice. This was in contrast to the studies of Pungsawat [61] and Koomsanit [70]

TABLE 13. QUALITIES OF NON-IRRADIATED AND IRRADIATED KAO NEAW SOM TOM DURING CHILLED STORAGE

Components	Quality attributes	Storage time (weeks)	Irradiation dose (kGy)		
			0	3	4
Steamed sticky rice	Moisture content (%)	0	42.63 ± 0.36a	42.63 ± 0.36a	43.75 ± 0.42a
		2	44.18 ± 0.38a	42.68 ± 0.09a	48.61 ± 0.36a
		4	42.97 ± 0.77b	42.25 ± 0.15b	45.69 ± 0.52b
		6	42.76 ± 0.37a	44.20 ± 0.81a	44.65 ± 0.53a
		8	43.57 ± 0.67b	46.18 ± 0.41b	43.01 ± 0.23b
	pH	0	5.94 ± 0.03a	5.85 ± 0.02a	5.85 ± 0.02a
		2	5.45 ± 0.01b	5.91 ± 0.01b	6.02 ± 0.01b
		4	5.65 ± 0.01ab	6.01 ± 0.02ab	6.02 ± 0.01ab
		6	6.04 ± 0.05b	6.01 ± 0.05b	5.98 ± 0.03b
		8	5.96 ± 0.08a	6.01 ± 0.02a	5.97 ± 0.03a
	Lightness	0	67.58 ± 0.13a	69.80 ± 0.13a	73.73 ± 0.13a
		2	73.49 ± 0.03ab	71.63 ± 0.05ab	74.50 ± 0.03ab
		4	74.19 ± 0.05c	74.57 ± 0.07c	72.74 ± 0.12c
		6	75.01 ± 0.20ab	73.73 ± 0.14ab	72.84 ± 0.23ab
		8	74.06 ± 0.12bc	71.39 ± 0.06bc	73.99 ± 0.13bc
	Hardness	0	5249 ± 87a	6658 ± 111a	7477 ± 136a
		2	7447 ± 183b	8308 ± 217b	8635 ± 68b
		4	9276 ± 112c	8801 ± 293c	9626 ± 105c
		6	9219 ± 170c	8982 ± 188c	9661 ± 188c
		8	9358 ± 278c	10121 ± 297c	9120 ± 103c

TABLE 13. QUALITIES OF NON-IRRADIATED AND IRRADIATED KAO NEAW SOM TOM DURING CHILLED STORAGE (cont.)

Components	Quality attributes	Storage time (weeks)	Irradiation dose (kGy)		
			0	3	4
	Stickiness	0	3106 ± 59a	2312 ± 22a	2237 ± 58a
		2	2641 ± 68b	2148 ± 45b	2138 ± 26b
		4	2372 ± 75c	1704 ± 29c	2109 ± 22c
		6	2270 ± 72d	1818 ± 38d	1549 ± 32d
		8	1460 ± 23e	785 ± 59e	981 ± 6e
Roasted chicken	Lightness	0	37.14 ± 4.09a	41.58 ± 0.12a	44.18 ± 0.07a
		2	40.42 ± 0.01a	41.23 ± 0.08ab	51.21 ± 0.08ab
		4	46.10 ± 0.03ab	42.55 ± 0.20ab	42.95 ± 0.02ab
		6	49.45 ± 0.31ab	40.14 ± 0.14ab	42.63 ± 0.11ab
		8	44.02 ± 1.16b	50.74 ± 0.10b	45.88 ± 3.59b
	Hardness	0	4712 ± 50a	5482 ± 203a	6012 ± 208a
		2	5551 ± 112ab	5242 ± 255ab	4533 ± 72ab
		4	4198 ± 126a	5743 ± 208a	5917 ± 141a
		6	4633 ± 41b	4456 ± 74b	5240 ± 86b
		8	4892 ± 77ab	5566 ± 145ab	4814 ± 125ab
	TBA value	0	0.039 ± 0.002a	0.049 ± 0.011a	0.060 ± 0.002a
		2	0.055 ± 0.004b	0.059 ± 0.014b	0.061 ± 0.003b
		4	0.081 ± 0.002c	0.106 ± 0.012c	0.110 ± 0.007c
		6	0.115 ± 0.003d	0.123 ± 0.003d	0.136 ± 0.007d
		8	0.121 ± 0.009e	0.131 ± 0.001e	0.152 ± 0.010e
Papaya salad	pH	0	6.46 ± 0.01a	6.01 ± 0.01a	6.01 ± 0.01a
		2	6.36 ± 0.01b	5.55 ± 0.01b	5.37 ± 0.01b
		4	5.14 ± 0.01c	5.34 ± 0.01c	5.25 ± 0.00c
		6	4.86 ± 0.04cd	5.12 ± 0.01cd	5.03 ± 0.02cd
		8	4.20 ± 0.02d	4.99 ± 0.02d	5.16 ± 0.01d

TABLE 13. QUALITIES OF NON-IRRADIATED AND IRRADIATED KAO NEAW SOM TOM DURING CHILLED STORAGE (cont.)

Components	Quality attributes	Storage time (weeks)	Irradiation dose (kGy)		
			0	3	4
	Lightness	0	64.65 ± 3.21c	64.23 ± 0.04c	61.73 ± 0.05c
		2	63.08 ± 0.06bc	63.60 ± 0.00bc	60.45 ± 0.06bc
		4	64.84 ± 0.03bc	60.94 ± 0.03bc	63.61 ± 0.11bc
		6	65.07 ± 0.08b	58.13 ± 0.08b	60.90 ± 0.09b
		8	64.79 ± 0.13a	57.53 ± 0.03a	55.90 ± 0.01a

Note: Means for each characteristic followed by the same letter in each sample during 8 weeks of storage are not significantly different at $p < 0.05$ by LSD test.

who reported a decrease in the moisture content of cooked rice and rice noodles during chilled storage. The pH of non- and irradiated steamed sticky rice had a fluctuating trend. For colour, there was no clear trend in changes of redness or yellowness of steamed cooked rice. However, irradiation increased lightness and a slight increase in this value was observed in the non-irradiated sample. Irradiation increased the hardness of sticky rice. This was different from the report of Sirisoontaralak and Noomhorm [74] who reported a decrease in the cooked rice hardness after irradiation. This might be due to a difference in the amylose and amylopectin ratio and also their structures. All samples showed an increase in the hardness value during storage under chilled conditions from retrogradation, although less increase was observed in a high dose irradiated sample. Stickiness decreased, depending on the irradiation dose. During chilled storage, it decreased in all samples.

4.4.2. Quality of roasted chicken

The changes in the redness and yellowness values of roasted chicken after irradiation and during storage were hardly perceptible. Lightness increased after irradiation and it increased during chilled storage (Table 13). Irradiation also increased the hardness of roasted chicken and it varied with doses, although it remained almost constant during storage. From the TBA values, irradiation induced more lipid oxidation and this increased significantly ($p < 0.05$) during chilled storage. This proved that irradiation induced more lipid oxidation in meat products [50].

4.4.3. Quality of papaya salad

As is apparent from Table 13, irradiation did not affect the pH of the papaya salad, although it decreased significantly ($p < 0.05$) with storage time. This might be due to microbial spoilage. Lightness decreased after irradiation, especially at a high dose (4 kGy), and it decreased significantly ($p < 0.05$) in the irradiated sample during chilled storage whereas that of the non-irradiated sample was unchanged.

4.4.4. Microbial quality of irradiated kao neaw som tom

From Table 14, steamed sticky rice was microbiologically safe during chilled storage for 8 weeks when irradiated at 3 and 4 kGy, while the non-irradiated sample was spoiled before 8 weeks. Roasted chicken had a shorter shelf life than sticky rice because it was protein rich. It was spoiled before 6 weeks under chilled condition but irradiation at 3 and 4 kGy could extend its shelf life to longer than 8 weeks. Among the three components, papaya salad was the most susceptible to microbial spoilage. A non-irradiated sample was spoiled before 2 weeks, but irradiation was effective in extending its shelf life to more than 8 weeks. When considering all the components of kao neaw som

TABLE 14. TOTAL PLATE COUNTS OF NON-IRRADIATED AND IRRADIATED KAO NEAW SOM TOM DURING CHILLED STORAGE

Components	Irradiation dose (kGy)	Population density (ln CFU/g)				
		Storage time (weeks)				
		0	2	4	6	8
Steamed sticky rice	0	3.04	5.00	6.31	6.88	7.58
	3	<1.00	<1.00	<1.00	<1.00	1.04
	4	<1.00	<1.00	<1.00	<1.00	<1.00
Roasted chicken	0	5.42	6.09	6.09	7.67	7.63
	3	<1.00	<1.00	<1.00	<1.00	1.00
	4	<1.00	<1.00	<1.00	<1.00	<1.00
Papaya salad	0	5.41	7.62	>7.00	>7.00	>7.00
	3	<1.00	<1.00	<1.00	<1.00	2.58
	4	<1.00	<1.00	<1.00	<1.00	<1.00

tom, the shelf life under chilled conditions could be extended from 2 weeks for untreated samples to more than 8 weeks using irradiation at 3 or 4 kGy.

4.4.5. Sensory quality of irradiated kao neaw som tom

There were no differences in the appearance, cohesiveness, softness and colour scores of non- and irradiated steamed sticky rice during chilled storage (Table 15). Panellists gave slightly lower scores for flavour and taste of irradiated sticky rice and this resulted in lower overall acceptability. These scores were almost unchanged during storage.

For roasted chicken, scores for all sensory attributes (appearance, colour, texture, flavour and taste) were not different among non- and irradiated samples at 3 and 4 kGy (Table 16). The scores did not change much during

TABLE 15. SENSORY QUALITY OF NON-IRRADIATED AND IRRADIATED STEAMED STICKY RICE DURING CHILLED STORAGE

Dose (kGy)	Storage time (weeks)	Appearance	Cohesiveness	Softness	Colour	Flavour	Taste	Overall acceptability
0	0	7.10a	6.08a	6.40a	7.30a	6.70bc	7.10b	7.20a
	2	6.90a	7.00a	7.00a	7.60a	7.20ab	7.30ab	7.20a
	4							
	6							
	8							
3	0	6.40a	6.30a	5.50a	6.30a	6.00bc	5.50b	5.90a
	2	6.70a	6.10a	6.60a	7.40a	6.80ab	6.90ab	6.70a
	4	7.00a	5.90a	6.40a	7.40a	6.30c	6.50b	6.50a
	6	6.90a	6.80a	6.40a	7.30a	6.90ab	6.50b	6.90a
	8	7.20a	6.40a	6.90a	7.50a	7.30a	7.30a	6.70a
4	0	7.50a	6.10a	6.30a	7.30a	6.80bc	6.70b	6.80a
	2	7.30a	7.00a	6.70a	7.80a	6.70ab	6.30ab	6.40a
	4	6.90a	6.20a	5.80a	7.20a	6.00c	6.10b	6.50a
	6	7.50a	6.10a	6.30a	7.30a	6.80ab	6.70b	6.80a
	8	6.90a	6.90a	6.80a	7.50a	7.20a	7.40a	7.10a

Note: Means for each characteristic followed by the same letter in each sample during 8 weeks of storage are not significantly different at $p < 0.05$ by LSD test.

TABLE 16. SENSORY QUALITY OF NON-IRRADIATED AND IRRADIATED ROASTED CHICKEN DURING CHILLED STORAGE

Dose (kGy)	Storage time (weeks)	Appearance	Texture	Colour	Flavour	Taste	Overall acceptability
0	0	6.80b	7.50a	6.80a	6.80b	7.00a	7.30a
	2	7.80a	7.70a	7.20a	7.30a	7.20a	7.70a
	4	7.30ab	7.20a	7.10a	7.70ab	7.30a	7.50a
	6						
	8						
3	0	7.00b	8.00a	7.00a	6.60b	7.50a	7.60a
	2	7.40a	7.10a	6.80a	7.50a	7.50a	7.30a
	4	6.80ab	6.70a	6.60a	7.20ab	6.70a	6.80a
	6	7.50ab	7.10a	7.20a	7.40ab	7.20a	7.40a
	8	7.40ab	6.70a	7.20a	7.40a	7.30a	7.10a
4	0	7.10b	6.80a	6.90a	6.70b	6.80a	6.90a
	2	7.50a	6.50a	7.00a	7.50a	6.70a	7.10a
	4	7.20ab	6.60a	7.00a	7.00ab	6.60a	6.90a
	6	7.30ab	6.60a	6.90a	6.90ab	7.00a	6.90a
	8	7.00ab	7.60a	7.20a	7.70a	7.60a	7.40a

Note: Means for each characteristic followed by the same letter in each sample during 8 weeks of storage are not significantly different at $p < 0.05$ by LSD test.

storage. Panellists still accepted irradiated steamed sticky rice and roasted chicken after 8 weeks of storage. The sensory quality of papaya, however, was severely affected by irradiation. Although panellists could not detect the difference in appearance of the non- and irradiated samples, they rejected the irradiated papaya salad because of the soft texture and impaired taste, even at the very low dose of 1 kGy (Table 17).

5. CONCLUSIONS

To obtain the longer shelf life of Thai spicy chicken basil rice (kao ka pao kai), cooked rice was prepared to obtain a harder texture and irradiated at 2 kGy. Three components: cooked chicken, sauce and blanched basil leaf

TABLE 17. SENSORY QUALITY OF NON-IRRADIATED AND IRRADIATED PAPAYA SALAD DURING CHILLED STORAGE

Dose (kGy)	Storage time (weeks)	Appearance	Texture	Colour	Taste	Flavour	Overall Acceptability
0	0	6.90a	7.70a	6.90a	7.40a	7.40a	7.70a
	1	7.00a	7.50a	7.30a	7.00a	6.90b	7.50b
1	0	7.00a	4.00a	6.90a	6.30a	6.90a	5.60a
	1	5.90a	3.40a	5.70a	4.90a	5.70b	3.90b

Note: Means for each characteristic followed by the same letter in each sample during a week of storage are not significantly different at $p < 0.05$ by LSD test.

were packed separately and irradiated at 2 kGy and at 0.1 kGy for the basil leaves. Regarding sensory quality, irradiated cooked rice had a longer shelf life of around 4 weeks. The shelf life of irradiated spicy chicken packed separately (>4 weeks) was much longer than the control sample (2 weeks). However, there is a need to preserve basil leaf because it was microbiologically spoiled by the second week of storage. For the stir-fried rice noodles with dried shrimp (pad Thai), irradiation at 3 kGy was not enough to eliminate *L. monocytogene*s entirely, which showed recovery after 2 weeks of chilled storage. Therefore, a dose of 4 kGy was recommended because the product was free from pathogens, safe from microbial spoilage and had acceptable sensory quality. After irradiation at 4 kGy, the shelf life of pad Thai could be extended to more than 4 weeks when storing under chilled conditions compared with a normal chilled ready meal, which has a shelf life of 5–7 d. For steamed sticky rice, roasted chicken and papaya salad (kao neaw som tom), a dose of 3 kGy was enough to control *L. monocytogenes* and *E. coli* during chilled storage. After irradiation at 3 kGy or more, the product was microbiologically safe when stored for more than 8 weeks, whereas the non-irradiated sample was spoiled by the second week of storage. Considering the sensory quality, panellists accepted irradiated steamed sticky rice and roasted chicken stored for 8 weeks under chilled conditions, but they rejected irradiated papaya salad owing to the soft texture and impaired taste.

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EFFECT OF GAMMA IRRADIATION ON THE QUALITY OF READY MEALS AND THEIR MEAT COMPONENTS

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Abstract

The effects of gamma irradiation on the quality of a chicken masala ready meal, minced red meat and salmon meat were studied in a series of experiments. Chicken masala meals treated with a dose of 1–3 kGy and stored for up to 14 d at refrigeration temperatures were analysed for vitamins E and B_1, oxidative rancidity, microbiological quality and 2-alkylcyclobutanones. It was found that vitamin levels were significantly reduced by a combination of irradiation, storage and reheating, while thiobarbituric acid (TBA) values decreased upon irradiation, which was an unexpected result. The shelf life of the meals was extended by irradiation as levels of bacteria were significantly reduced in the prepared meals upon treatment. Both 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone were detected in the irradiated samples, thereby showing that these compounds can be used as markers of irradiation treatment for such food products. In order to study potential components for ready meals, freshly minced beef was used to prepare beef patties, which were vacuum packed and treated with doses from 2.5 to 10 kGy. Following irradiation, half the samples were retained in vacuum packs while the remainder were transferred to sterile containers and overwrapped with cling film. Samples were stored for up to 21 d at refrigeration temperature with TBA values and microbiological quality being analysed at regular intervals. The TBA values of vacuum packed samples were unaffected by irradiation although storage did result in an increase. On the other hand, the TBA values of overwrapped patties increased upon irradiation and storage. The microbiological quality of the irradiated patties upon storage was significantly better than those of untreated patties. A further series of experiments were undertaken to determine the effect of adding antioxidants to beef and salmon patties in order to improve lipid stability upon irradiation. In an initial experiment, minced beef patties were prepared with the antioxidants α-tocopherol, epicatechin, quercetin and resveratrol included at concentrations of 110 and 550 μmol/kg, after which they were overwrapped and irradiated with 2.5 and 5 kGy. Following irradiation, the patties were stored for up to 28 d at refrigeration temperature and TBA values measured at intervals. Inclusion of the antioxidants reduced lipid oxidation, with the patties containing resveratrol having the lowest TBA values. Samples containing 550 μmol/kg of antioxidant had lower TBA values than those with 110 μmol/kg. Further

experiments were undertaken to study the effect of including the same antioxidants at concentrations of 25, 50, 250, 500 and 700 μmol/kg in minced beef and salmon meat patties irradiated at 2.5 kGy and stored for up to 14 d at refrigeration temperature. As noted previously, resveratrol was the most effective antioxidant for improving lipid stability in minced beef patties while the lowest TBA levels for salmon meat patties were obtained for those containing epicatechin. Overall, concentration had a significant effect on the TBA values with lower values being obtained for those samples containing the higher inclusion levels.

1. INTRODUCTION

The prepared convenience foods sector in Ireland is a significant part of the Irish economy. In 2001, just under half of the sector's total output was exported for a value of €841 million, representing a 12% annual increase [1]. The sector's strong growth, both in exports and in total sales, has made it one of the fastest growing sectors of the food industry in Ireland [2]. Work carried out by de Boer et al. [2] confirmed that perceived time pressures contributed positively to the purchase of both ready meals and takeaway meals, which was in line with previous findings of Verlegh and Candel [3] who found that time pressure had a positive effect on the frequency of consumption of TV dinners and prepared dinners, meal centres and takeaways. Other reasons which contributed positively to the purchase of ready meals included not enjoying cooking for oneself, value-for-money perception of convenience foods and different eating times of family members [2]. As a consequence of the increased market for convenience foods, and particularly ready meals, the food industry is interested in ways of producing meals which are safe to eat, nutritious and with an acceptable shelf life. One technology investigated as a means of achieving these objectives is food irradiation [4].

The results of previous research carried out in Belfast [5–8] concluded that using an irradiation dose of 2 kGy would extend the shelf life of refrigerated ready meals, whole meals (e.g. roast pork, boiled potatoes, mixed vegetables) and pureed meals (e.g. pureed chicken and gravy, boiled potatoes, garden peas) for up to 14 d. However, the importance of storing the meals under suitable refrigeration conditions (<3°C) was highlighted in order to obtain maximum benefit from the irradiation treatment. The research showed that vitamin losses due to treatment with ionizing irradiation were comparable to those induced by cooking and storage. From a regulatory point of view, results obtained in the experimental work showed that both the 2-alkylcyclobutanone (EN1785) and ESR spectroscopy (EN1787) standard detection methods could be used to identify irradiated products, with EN1785

being carried out on the meat component of the meals and EN1787 using the packaging as the radiation marker [9].

The following work is a further extension of that carried out to date. The studies reported here focus primarily on the meat components of ready meals, such as minced beef and salmon meat. Some initial work was undertaken on ready meals, but as sourcing of these meals proved difficult extensive studies could not be undertaken. The experimental work studied the effects of ionizing radiation on the quality attributes of the meals important to the consumer, such as microbiological quality and off flavours as measured in terms of oxidative rancidity and nutritional quality.

2. MATERIALS AND METHODS

2.1. Effect of irradiation on the quality of a chilled chicken masala ready meal

2.1.1. Experimental protocol

A batch of chilled chicken masala ready meals was obtained from a local manufacturer in Northern Ireland. The meals were obtained from the processor within 2 h of production and brought back to the laboratory where they were stored at 3 ± 1°C until irradiated (Section 2.5.1). The samples were analysed for thiamine (Section 2.5.2) and α-tocopherol (Section 2.5.3), oxidative rancidity (Section 2.5.4), microbiological quality and 2-alkylcyclobutanones (Section 2.5.5) in order to determine if irradiation treatment could be confirmed.

The vitamin and oxidative rancidity analyses were carried out on the same samples. A total of 72 meals were given doses of 1, 2 or 3 kGy or left non-irradiated to serve as controls, thereby giving 18 meals per treatment. Of the 18 meals within each batch, six were analysed immediately while six were stored for 7 d and six for 14 d at 3 ± 1°C. Upon analysis, three meals at each dose level were analysed in the fresh state while the other three meals were reheated in a convection oven at 180°C for 20 min to give an internal temperature of 80°C. The meals were allowed to cool to ambient temperature prior to being homogenized by a food processor and analysed.

For the microbiological analysis, a total of 64 samples from the same batch of meals were given the same irradiation doses as those described previously, with 16 meals being treated per dose. Within each batch of 16, four meals were analysed at each storage time of 0, 7, 11 and 14 d, two being stored at 3 ± 1°C and two at 10 ± 1°C. These meals were not reheated prior to analysis. The samples were homogenized in a stomacher for 2 min prior to sampling. On

each sampling day, the meals were assessed as follows: total bacteria count (TBC) (30°C for 48 h) on typtone soya agar containing 0.6% yeast extract (TSAYE); coliform count (37°C for 18–24 h) on McConkey No. 3 agar; and psychrotrophic count of TSAYE (7°C for 10 d).

2.2. Effect of irradiation on the quality of minced beef patties

2.2.1. Effect of irradiation on the oxidative rancidity and microbiological quality of vacuum packed and overwrapped minced beef patties stored for 21 d at 3°C

On each of three separate occasions, freshly minced beef (15% lipid) was obtained from a local butcher in Belfast. Under aseptic conditions 65 patties, each weighing 50 g, were produced and vacuum packed. The samples were irradiated with doses of 2.5, 5, 7.5 and 10 kGy (Section 2.5.1) or left non-irradiated to serve as controls, thereby giving 13 samples at each dose. Following irradiation, 35 samples were retained within the vacuum packs while the other 30 were removed aseptically from the packaging in a laminar flow cabinet, placed in sterile cartons and covered in cling film in order to give 30 overwrapped samples. Five samples were either analysed immediately (day 0) and five at each treatment after 1, 2, 5, 7, 14 and 21 d at 3 ± 1°C for microbiological quality, or after 1, 2, 5, 7, 14 and 21 d at 3 ± 1°C for oxidative rancidity, thereby giving two samples per treatment.

The same patties were used for both the oxidative rancidity and microbiological assessment, the samples for microbiological analysis being taken under aseptic conditions prior to the patties being analysed for oxidative rancidity (Section 2.5.4).

For microbiological quality, on each sampling day, the vacuum packed samples were assessed as follows: TBC (30°C for 48 h) on TSAYE; anaerobic count (35°C for 48 h) on TSAYE; coliform count (37°C for 18–24 h) on McConkey No. 3 agar; psychrotrophic count (7°C for 10 d) on TSAYE; *Pseudomona* spp. (25°C for 48 h) on *pseudomonad* selective agar (PSA) to CFC formulation; and lactic acid count (30°C for 48 h) on deMan, Rogosa, Sharpe (MRS) agar using pour plates with an overlay. In the case of the overwrapped samples, total viable, psychrotrophic, *pseudomona* and coliform counts were carried out.

2.3. Effect of ionizing radiation and storage on the oxidative rancidity of minced beef patties containing natural antioxidants at different concentrations

2.3.1. Experimental protocol 1

Freshly minced beef (6% lipid) was purchased from a local supplier in Belfast and transferred to the laboratory where it was thoroughly mixed to provide a homogenous bulk sample. This was used to prepare a total of 120 patties, each weighing 50 g, containing the antioxidants α-tocopherol, epicatechin, quercetin and resveratrol at concentrations of 110 and 550 μmol/kg or with no antioxidant added to serve as controls. Following preparation, each pattie was placed in a plastic container, which was double wrapped with cling film. The samples were irradiated at doses of 2.5 and 5 kGy or left non-irradiated. The samples were stored for periods of 1, 7, 14, 21 and 28 d at 4 ± 1ºC, after which they were analysed for oxidative rancidity using the thiobarbituric acid (TBA) method (Section 2.5.4). The experiment was repeated on three separate occasions.

2.3.2. Experimental protocol 2

As previously, fresh minced beef (8% lipid) was purchased and mixed to provide a homogenous bulk sample. On this occasion 100 patties, each weighing 50 g, were prepared. The antioxidants α-tocopherol, epicatechin, quercetin and resveratrol were included at concentrations of 25, 50, 100, 250, 500 and 700 μmol/kg whilst other patties were prepared with no antioxidant. The samples were packed as before and treated with an irradiation dose of 2.5 kGy or left non-irradiated. They were then stored for 1 and 14 d at 4 ± 1ºC, pending oxidative rancidity analysis (Section 2.5.4). The experiment was repeated on three separate occasions.

2.4. Effect of ionizing radiation and storage on the oxidative rancidity of salmon meat patties containing natural antioxidants at different concentrations

2.4.1. Experimental protocol

On each of three separate occasions, fresh skinless sides of salmon (15% lipid) were obtained from a local supplier in Belfast and transferred to the laboratory. The salmon was passed through a mincer and the resultant puree was thoroughly mixed to provide a single homogenous bulk sample. This was

used to prepare 100 salmon patties, each weighing 50 g. As previously carried out for minced beef (Section 2.3.2), the antioxidants α-tocopherol, epicatechin, quercetin and resveratrol were included at concentrations of 25, 50, 100, 250, 500 and 700 μmol/kg or no antioxidant was added to serve as controls, after which they were packed, irradiated and analysed as described previously.

2.5. Irradiation treatment and analytical methods

2.5.1. Irradiation treatment

Irradiation was carried out using ^{60}Co as the source of ionizing radiation (Gammabeam-650, MDS Nordion, Kanata, Canada). Gammachrome YR dosimeters (AEA Technology, Harwell, United Kingdom) were placed on samples receiving a dose of 2 kGy or less while amber dosimeters (AEA Technology, Harwell, UK) were placed on those samples receiving 2.5 kGy or greater. Following irradiation, the change in absorbance of the dosimeters was measured using a Unicam SP-8 uv/vis spectrophotometer (Unicam, Cambridge, UK). Thickness of dosimeters was measured using a digital electronic micrometer (RS Components, Corby, UK) and the recorded absorbances were corrected to a standard thickness of 3 mm. The corresponding dose received was obtained from a calibration graph provided by the National Physical Laboratory (Teddington, UK).

2.5.2. Thiamine (vitamin B_1) determination

Thiamine concentration of the ready meals was measured using a modification of the HPLC method of Finglas and Faulks [10], as outlined by Graham et al. [11]. Each sample was analysed in duplicate.

2.5.3. α-tocopherol (vitamin E) determination

Determination of α-tocopherol was achieved using HPLC after saponification and extraction from the ready meal samples into *n*-hexane according to the method of McMurray and Blanchflower [12] and McMurray et al. [13] with slight modifications. To a 1 g aliquot of extracted oil, 1 mL of 60% potassium hydroxide and 5 mL of 50% pyrogallol in ethanol were added, the mixture flushed with nitrogen gas, heated to boiling and allowed to simmer for 20 min. After cooling, 2 mL water was added and the tubes shaken, followed by the addition of 3 mL hexane and vigorous shaking for 1 min. The mixture was then centrifuged at 2500g for 30 min. The top solvent layer containing the vitamin was removed and evaporated to dryness under nitrogen and reconstituted in

200 μL of acetone prior to HPLC analysis. The HPLC equipment used for analysis consisted of a Hewlett Packard HP 1090 M liquid chromatograph with integral fluorescence detector, autosampler and autoinjector along with a Hewlett Packard 9000 series 320 data station (Winnersh, Wokingham, UK). The HPLC column used was 250 mm × 4.6 mm i.d. packed with 10 μm spherisorb ODS 2 (Phase Separations, UK). The mobile phase was methanol:water in a 97:3 ratio, the flow rate was 1.8 mL/min while the injection volume was 20 μL. The column temperature was maintained at ambient. Fluorescence detection was performed with an excitation wavelength of 289 nm and an emission wavelength of 335 nm. Identification and quantification of α-tocopherol was performed by comparing the sample retention times and peak areas with those of external standards. The concentration of α-tocopherol was expressed as milligrams per gram of sample on a fresh weight basis. Recoveries of α-tocopherol were checked by spiking samples with standard solutions of α-tocopherol and treating as for the meal samples.

2.5.4. Oxidative rancidity measurement (TBA method)

Oxidative rancidity of the samples was determined by measuring their TBA values according to the method of Pikul et al. [14], but using trichloroacetic acid (TCA) in place of perchloric acid owing to the hazards associated with perchloric acid. A 10 g portion of sample was homogenized along with 35 mL of 5% TCA and 1 mL of 7.2% butylated hydroxyanisole in ethanol for 3 min using a Polytron PT 2000 homogenizer (Kinematica, Lucerne, Switzerland). The resultant extract was filtered through a Whatman No. 541 filter paper (Whatman International, Maidstone, UK), collected in a 50 mL volumetric flask and made to volume with 5% TCA. A 5 mL aliquot of extract was pipetted into a glass tube followed by 5 mL of TBA, which had been freshly prepared by dissolving 0.2883 g of 2-thiobarbituric acid (Sigma Chemical, Poole, UK) in 100 mL of distilled water. The tubes were stoppered and the contents well mixed prior to heating for 60 min in a boiling water bath (Grant Instruments, Royston, UK). A blank was prepared in a similar manner using 5 mL of TCA and 5 mL of TBA. The tubes were removed from the water bath, cooled in water for 10 min and the absorbance (D) of their contents measured in a UV500 spectrophotometer (Unicam, Cambridge, UK) at 538 nm using 1 cm cells. The TBA value was calculated as:

$$\text{TBA value (as milligrams of malonaldehyde per kilogram of sample)} = (D_{\text{sample}} - D_{\text{blank}}) \times \text{K}$$

where K is a constant coefficient specifically calculated for individual products.

2.5.5. 2-alkylcyclobutanone identification

The cyclobutanones, dodecylcyclobutanone (DCB) and tetradecylcyclobutanone (TCB) were determined according to European Standard EN1785:2003 [9].

2.6. Statistical analysis

The results for all the experimental protocols were subjected to analysis of variance using Genstat 5.

3. RESULTS

3.1. Effect of irradiation on the quality of a chicken masala chilled ready meal

3.1.1. Oxidative rancidity

Figure 1 shows the results of gamma irradiation on the oxidative rancidity of the ready meals as measured by the TBA method. Statistically, it was found that the irradiation dose had a highly significant ($p < 0.01$) effect on the TBA

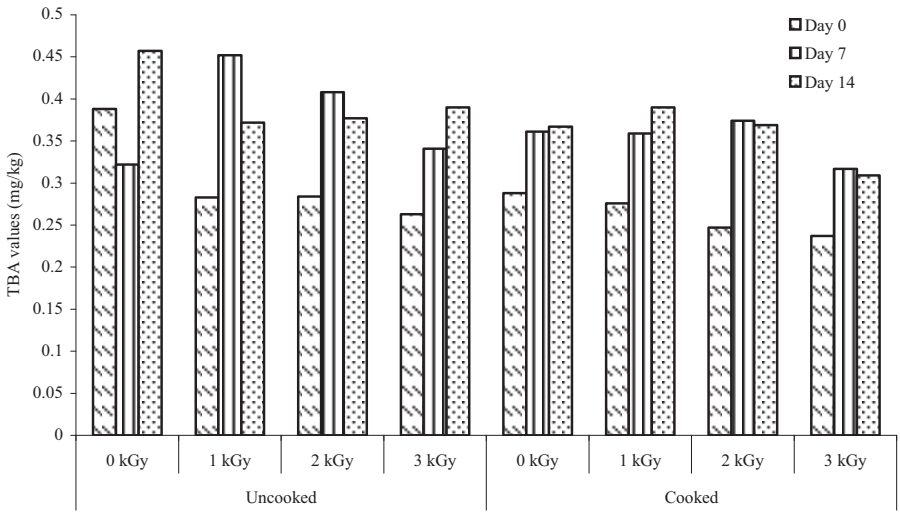

FIG. 1. Effect of irradiation on the TBA values (mg malonaldehyde/kg) of a chicken masala ready meal stored for up to 14 d at 3 ± 1°C (SEM = 0.0214, n = 3).

values of the meals. However, it can be seen that the actual values decreased with increasing irradiation dose over the range 0–3 kGy. The values obtained from the non-irradiated and 1 kGy samples were similar, with larger decreases being observed between 1 and 3 kGy. Overall, there was only a decrease of 13% in the TBA values between 0 and 3 kGy. This is not surprising as the meals contained only 5.2% lipid. Storage had a significant effect ($p < 0.001$), the TBA values with the most significant increase occurring between day 0 and day 7 of storage with little change taking place between days 7 and 14. Overall, reheating (cooked) the meals resulted in a 9% decrease in the TBA values. It is important to note that the values obtained were all less than unity, thereby indicating that irradiation of the meals would not contain off flavours due to oxidative rancidity.

The results obtained for oxidative rancidity were surprising as it was expected that the TBA values would increase on irradiation, storage and cooking. One possibility for this result is the presence of antioxidants in the meals, which were quenching the free radicals produced by the ionizing radiation. The meals proved to be a good source of α-tocopherol (Fig. 2) and contained a range of ingredients such as onion, tomato, red pepper and spices, which are known for their antioxidant activity. Another explanation could be that there is a preferential attack mechanism employed by the free radicals on the α-tocopherol and thiamine present in the meals, thereby reducing the

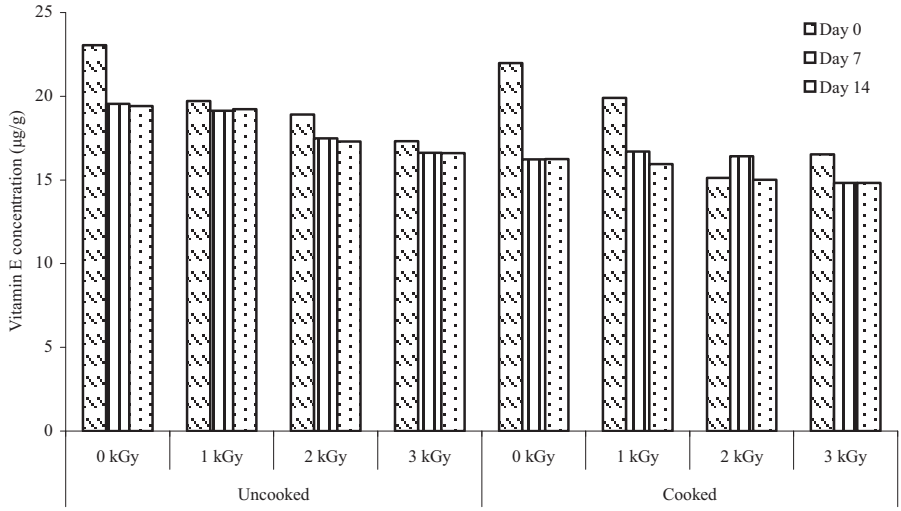

FIG. 2. Effect of irradiation on the α-tocopherol content (μg/g) of a chicken masala ready meal stored for up to 14 d at 3 ±1°C (SEM = 1.110, n = 3).

concentration of these vitamins. This consequently resulted in a decrease in the TBA values with an increase being observed in the amounts of α-tocopherol and thiamine destroyed.

3.1.2. α-tocopherol (vitamin E)

An α-tocopherol level of 23.1 μg/g was measured in the chicken masala meals prior to being irradiated, reheated or stored. It was found that the concentration of this fat soluble vitamin decreased linearly with increasing irradiation dose ($p < 0.001$). Taking an average value over all the treatments, vitamin E concentration in the meals decreased by 5, 14 and 17%, respectively, when treated with doses of 1, 2 and 3 kGy, compared with their non-irradiated counterparts (Fig. 2). The decreases observed were not unexpected as it is known that α-tocopherol is radiation labile, being the most sensitive of the fat soluble vitamins [15, 16]. Reheating the meals resulted in a further reduction in α-tocopherol concentration, with an overall decrease of 11% being observed.

Storage also resulted in a very highly significant ($p < 0.001$) reduction in the α-tocopherol content of the meals (Fig. 2), with the greatest diminution being found for the non-irradiated samples, where a 21% decrease between days 0 and 14 was observed. Reductions of 11, 5 and 7% were observed for the meals treated with 1, 2 and 3 kGy, respectively, between days 0 and 14. Generally, the greatest decrease in vitamin levels occurred between days 0 and 7, with the exception of the 2 kGy samples, in which case a reduction of 5% occurred between days 7 and 14.

3.1.3. Thiamine (vitamin B_1)

For thiamine (Fig. 3), an average concentration of 6.65 μg/g was measured in the non-irradiated chicken masala ready meal at day 0 of storage and prior to reheating, with average values in the non-irradiated samples after 7 and 14 d storage at refrigeration temperature being 5.72 and 5.64 μg/g, respectively. Irradiation of the meals with 1 kGy resulted in an overall decrease in thiamine levels of 16% compared with non-irradiated meals, with reductions of 21% and 31% being observed at dose levels of 2 and 3 kGy, respectively, in comparison to their non-irradiated counterparts.

Reheating the meals resulted in an overall reduction in thiamine content (Fig. 3) from 5.13 to 4.80 μg/g, which represented a decrease of only 6%. At the 3 kGy dose level, the thiamine content was the same for both the reheated and chilled meals. The results obtained were similar to those of other studies where irradiation and cooking have been shown to reduce thiamine levels [11, 17].

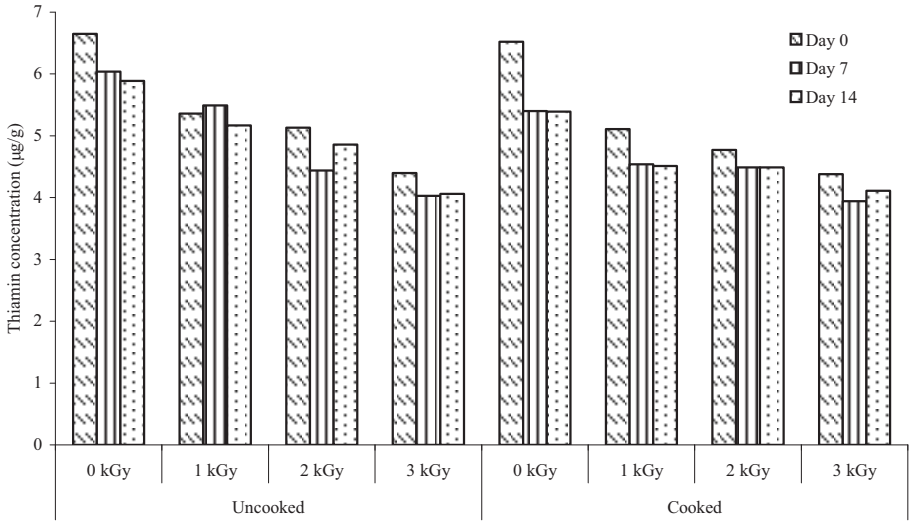

FIG. 3. Effect of irradiation on the thiamine content (μg/g) of a chicken masala ready meal stored for up to 14 d at 3 ±1°C (SEM = 0.0997, n = 3).

The thiamine content of the meals decreased with increasing storage time (Fig. 3). The greatest decrease in thiamine concentration was observed for the non-irradiated meals, where an overall decrease of 13% occurred between days 0 and 7, the levels decreasing only 1% further between days 7 and 14. The thiamine content of the meals given 1, 2 and 3 kGy decreased similarly by approximately 7% between days 0 and 14.

3.1.4. Alkylcyclobutanone identification

Both DCB and TCB were detected in the irradiated meals, although ions *m/z* 98 and 112 were not detected in all of the samples analysed. As expected from previous work [18, 19], irradiation had a very significant effect ($p < 0.001$) on the concentration of both DCB and TCB in the chilled meals, with higher levels of 2-DCB being detected compared with TCB. Levels of 0.071, 0.117 and 0.172 μg DCB/g lipid were detected in the chilled (non-reheated) meals for the 1, 2 and 3 kGy doses, respectively, while TCB concentrations of 0.014, 0.034 and 0.053 μg/g lipid were detected. Cooking or storage did not have a significant effect on the levels of the cyclobutanones as similar levels of cyclobutanones were measured for DCB in the cooked and uncooked meals (Fig. 4). Detection of both cyclobutanones proved possible for the chicken masala meals but the

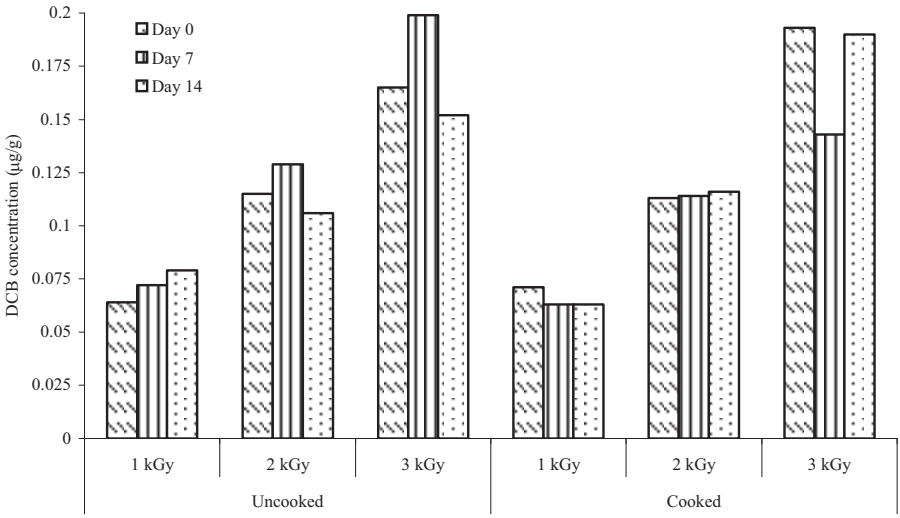

FIG. 4. Effect of irradiation on the 2-DCB content (µg/g lipid) of a chicken masala ready meal stored for up to 14 d at 3 ±1°C (SEM = 0.0114, n = 3).

method would need to be developed in order that ions *m/z* 98 and 112 can be detected for each sample in the correct ratios. It should also be borne in mind that as the meals had a lipid content of only 5%, detection of these compounds is more difficult than if the meals contained a higher amount of lipid.

3.1.5. Microbiological quality

The effects of irradiation on the microbiological quality of the chilled meals are illustrated in Figs 5 and 6. In all cases, irradiation significantly reduced the levels of bacteria in the chicken masala ready meals. Figure 5 shows the effect of irradiation and storage on the TBCs of the meals stored at 3 and 10°C. For the 3°C samples (Fig. 5(a)), all three doses reduced the viable bacteria levels in a similar manner, with numbers being significantly lower than the 0 kGy meals even after 14 d of storage. At 10°C storage (Fig. 5(b)), similar TBCs were observed for the non-irradiated, 1 and 2 kGy meals, with the 3 kGy dose significantly reducing levels over the 14 d storage period. The bacterial loads in the meals stored at 10°C were significantly higher than those stored at refrigeration temperature.

Figure 6 demonstrates the effects of irradiation and storage on the psychrotrophic counts for meals retained at the two storage temperatures. For meals stored at 3°C (Fig. 6(a)), similar levels of bacteria were found at the 0

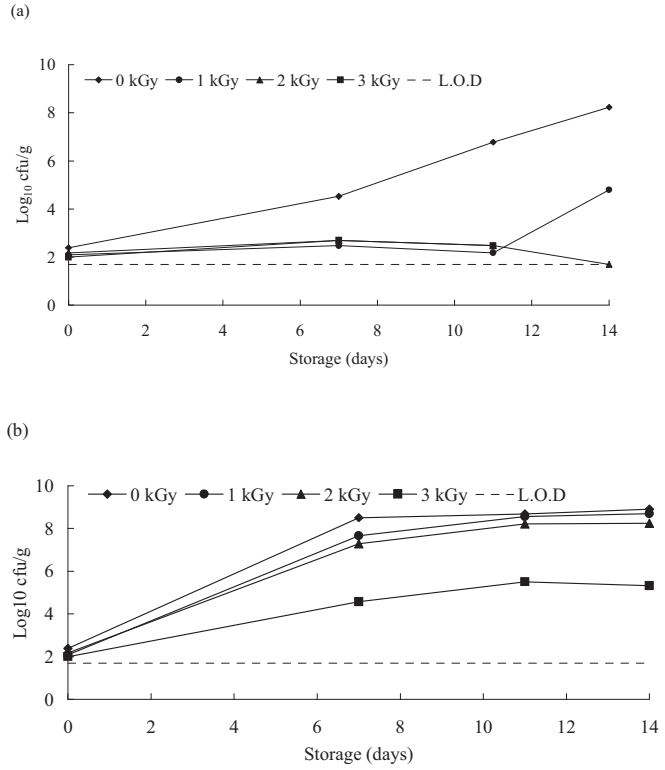

FIG. 5. Effect of irradiation and storage on the TBCs of a chicken masala ready meal stored at 3 ±1°C (a) and 10 ±1°C (b). LOD = limit of detection (1.70 ln CFU/g).

and 1 kGy dose levels while no bacteria were detected at 2 and 3 kGy, being below the LOD of 1.70 ln CFU/g. For the meals stored at 10°C (Fig. 6(b)), similar bacterial loads were observed for the non-irradiated samples and for those given doses of 1 and 2 kGy. Irradiation at 3 kGy had the most significant effect on the numbers of psychrotrophic bacteria, although it was noted that none were detected at day 0 of storage. Coliforms were not detected in any of the ready meals during storage.

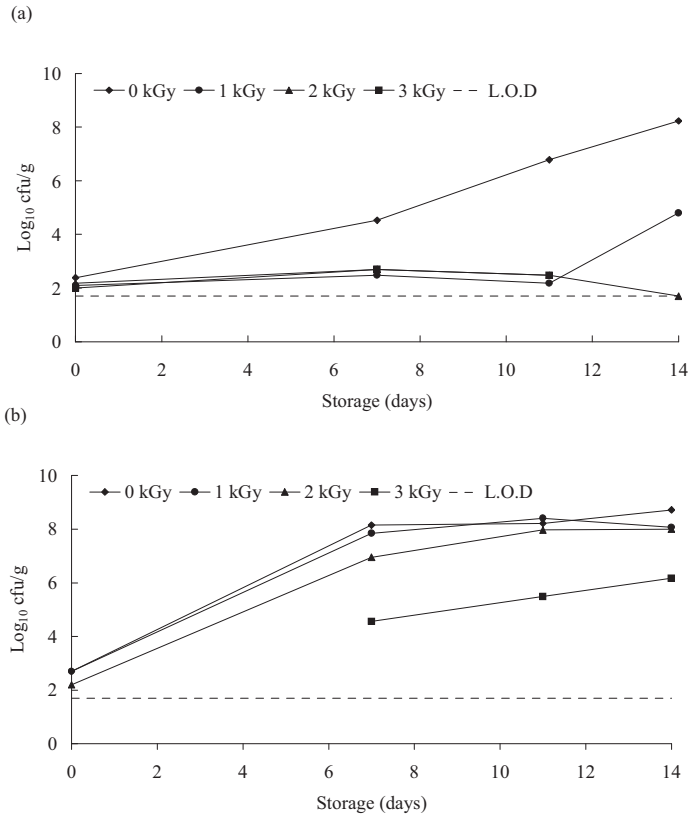

FIG. 6. Effect of irradiation and storage on the psychrotrophic counts of a chicken masala ready meal stored at 3 ±1°C (a) and 10 ±1°C (b). LOD = limit of detection (1.70 ln CFU/g).

3.2. Effect of irradiation on the quality of minced beef patties

3.2.1. Effects of irradiation on the oxidative rancidity and microbiological quality of vacuum packed and overwrapped minced beef patties stored for 21 d at 3°C

3.2.1.1. Oxidative rancidity

When the results for the vacuum packed samples were analysed statistically, it was found that irradiation dose did not have a significant effect on the TBA values. In comparison to the results obtained for the previous

experiments, it was found that there was an increase in the TBA values with increasing dose (Fig. 7). However, it was also found that the increase between the non-irradiated samples and those given 2.5, 5, 7.5 and 10 kGy was 13, 3, 6 and 14%, respectively, thereby indicating that the changes with dose were not consistent. Similar values were obtained for the 2.5 and 10 kGy samples.

Storage had a highly significant effect ($p < 0.001$) on the TBA values of the beef patties over the 21 d of storage. As can be seen from Fig. 7, the TBA values were similar from day 0 to 7 for 0 kGy and the 2.5 kGy patties increased from day 7 to 21. However, for the other doses, there was no particular trend in the values from day 0 to 21.

Figure 8 shows a comparison of the TBA values obtained for minced beef patties irradiated in vacuum packages followed by subsequent storage for 21 d, remaining in the vacuum packs or after being transferred to overwrapped packages. Overall, there was a significant increase in the TBA values with increasing irradiation dose and this was due to the increases that occurred in the overwrapped beef patties. A 39% increase in TBA values was observed between the overwrapped non-irradiated patties and those given 2.5 kGy, with increases of 57% and 70% being found in the non-irradiated samples and those given 5 and 7.5 kGy, respectively. The values obtained for the 7.5 and 10 kGy overwrapped samples were similar.

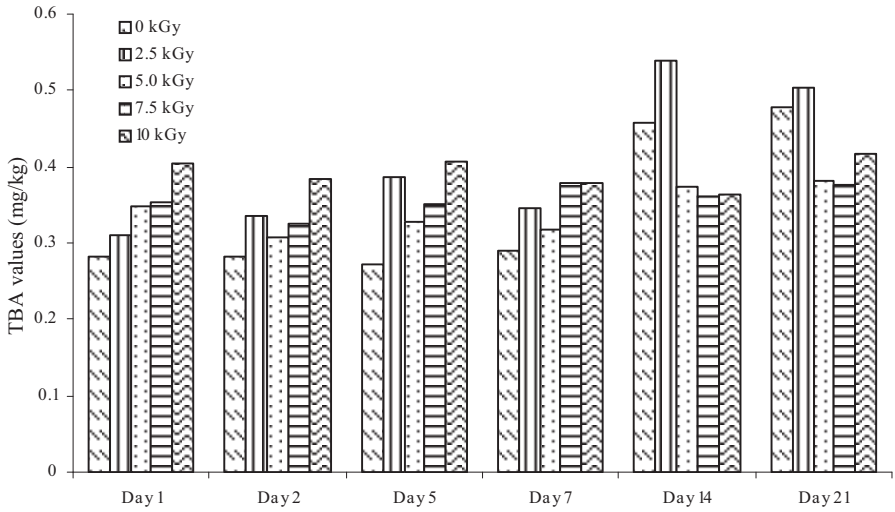

FIG. 7. Effect of irradiation on the TBA values (mg malonaldehyde/kg) of vacuum packed minced beef patties stored for up to 21 d at 3 ±1°C (SEM = 0.0476, n = 3).

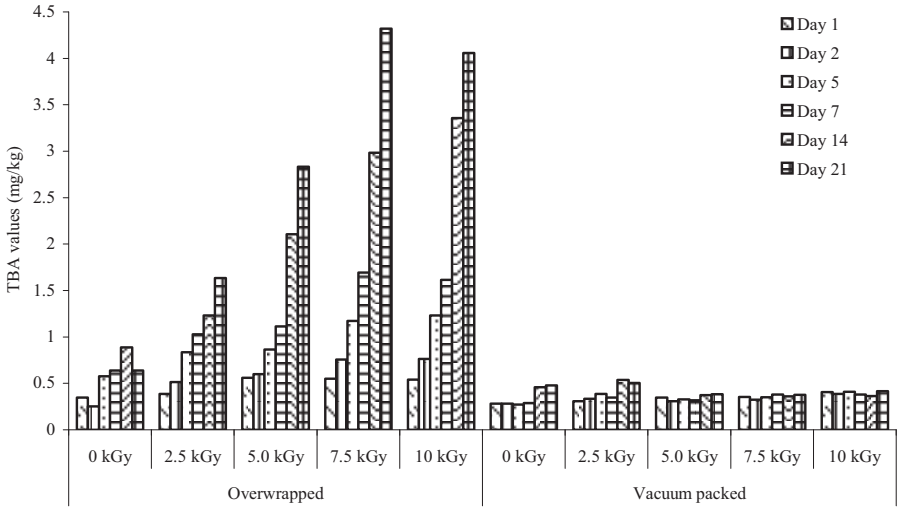

FIG. 8. Effect of irradiation on the TBA values (mg malonaldehyde/kg) of overwrapped and vacuum packed minced beef patties stored up to 21 d at 3 ±1 ℃ (SEM = 0.1389, n = 3).

The TBA values of the overwrapped samples increased with storage, although it was noted that little change occurred over the first two days of storage. If a value of unity is taken as the TBA value above which the meat would be deemed unacceptable from an organoleptic point of view, only the non-irradiated samples were acceptable over the 21 d storage period. The 2.5 and 5 kGy samples were acceptable up to 7 d, while the 7.5 and 10.0 kGy samples were acceptable for less than 5 d.

According to work reported by Ahn et al. [20] and Kim et al. [21], despite the intrinsic antioxidant activities of fresh meat, irradiation was found to accelerate lipid oxidation in raw pork and beef patties under aerobic conditions. Therefore, in order to extend the shelf life of products such as minced beef patties when stored in overwrapped packages, it may be beneficial to add antioxidants to the meat prior to irradiation in order to prevent or slow down the process of oxidative rancidity. On the other hand, the patties could be irradiated and stored in vacuum packs in order to prevent the occurrence of oxidative rancidity as noted previously. Results of this work show that the average TBA value obtained for the overwrapped samples was 1.34 mg malonaldehyde/kg compared with a value of 0.37 mg/kg for the vacuum packed samples.

3.2.1.2. Microbiological quality

Both irradiation and storage significantly affected the microbiological quality of both the overwrapped and vacuum packed beef patties. Figure 9 clearly shows that irradiation reduced the TBCs in samples stored in both types of packaging, although it should be noted that higher counts were obtained for the overwrapped samples (Fig. 9(b)) compared with those stored in vacuum packages (Fig. 9(a)). Irradiation doses greater than 2.5 kGy reduced the bacterial levels to below the LOD in the vacuum packed samples, with all doses reducing detectable numbers in the overwrapped patties.

There was little change in the TBCs over the 21 d storage period in the vacuum packed samples, with numbers increasing steadily in the non-irradiated overwrapped samples. Similar results were obtained for the pseudomonds

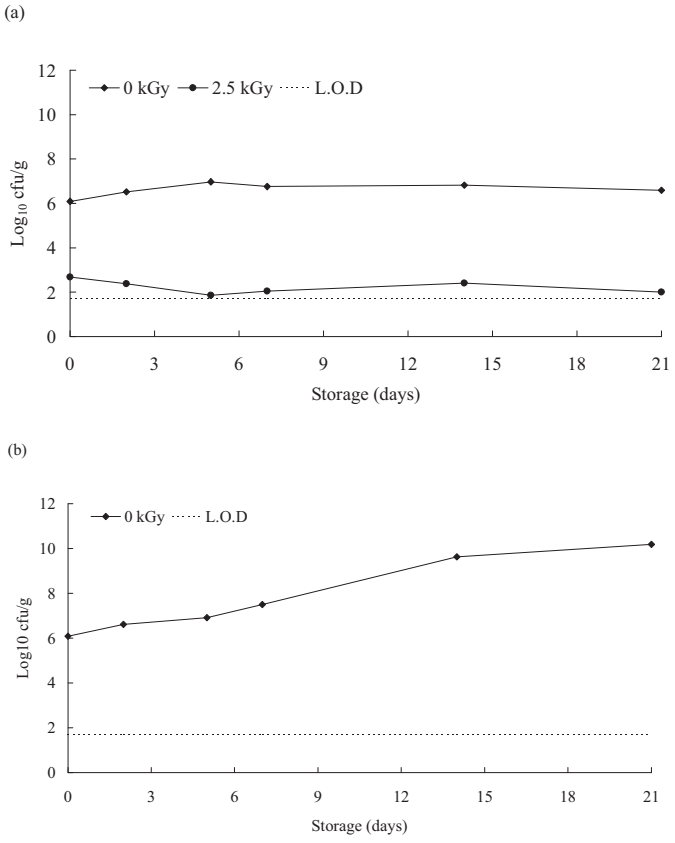

FIG. 9. Effects of irradiation treatment and storage on the TBCs of vacuum packed (a) and overwrapped (b) minced beef patties stored at 3 ±1°C. LOD = limit of detection (1.7 ln CFU/g).

counts. The levels of psychrotrophic bacteria counted in the beef patties are presented in Fig. 10. Irradiation significantly reduced the numbers of psychrotrophs in both types of packaging, although the bacteria appeared to be more sensitive in the overwrapped packs (Fig. 10(b)) owing to the availability of oxygen. Similar bacterial numbers were detected at the 2.5 and 5 kGy doses in the vacuum packages (Fig. 10(a)), while bacteria were only detectable at the 2.5 kGy dose in the overwrapped samples. As for the TBCs, the levels of bacteria were constant over the 21 d of storage in the vacuum packed patties, while a steady increase was observed in the overwrapped samples.

Anaerobic bacteria (Fig. 11) were detected only in the non-irradiated and 2.5 kGy beef patties, with similar levels being detected over the storage period at 3°C. The irradiation doses of 5–10 kGy reduced the levels of these bacteria to

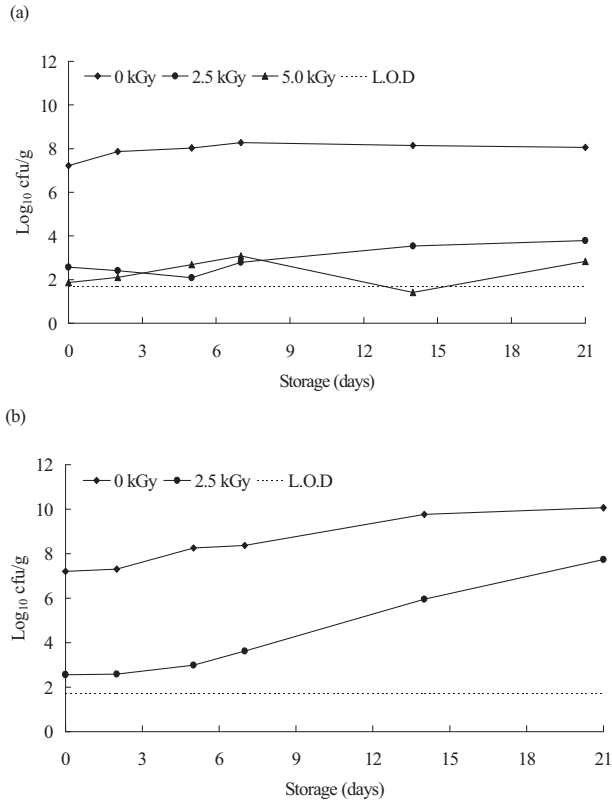

FIG. 10. Effects of irradiation treatment and storage on the psychrotrophic counts of vacuum packed (a) and overwrapped (b) minced beef patties stored at 3 ± 1°C. LOD = limit of detection (1.7 ln CFU/g).

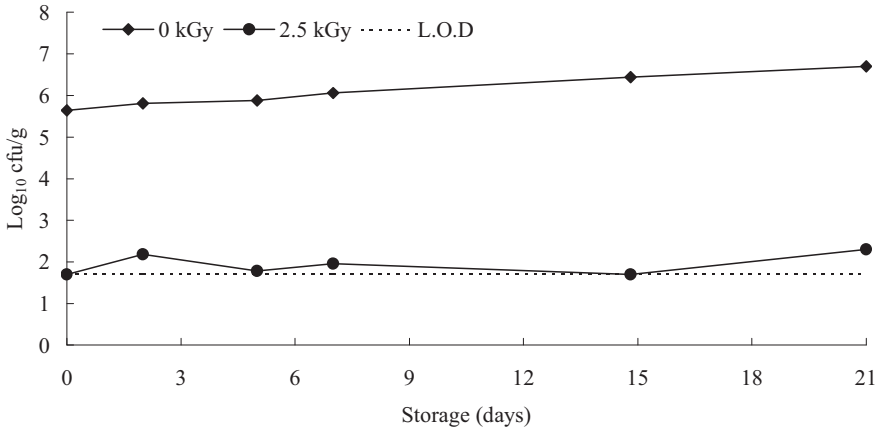

FIG. 11. Effects of irradiation and storage on the anaerobic bacteria in vacuum packed minced beef patties stored at 3 ±1°C. LOD = limit of detection (0.70 ln CFU/g).

below the limit of detection. Irradiation significantly reduced the numbers of lactic acid bacteria (Fig. 12) in the vacuum packed patties, with 7.5 and 10 kGy reducing levels to below the detectable limit. Similar numbers were observed for the 2.5 and 5 kGy irradiation treatments. The levels of bacteria detected in the non-irradiated and irradiated samples remained similar over the 21 d of storage at 3°C.

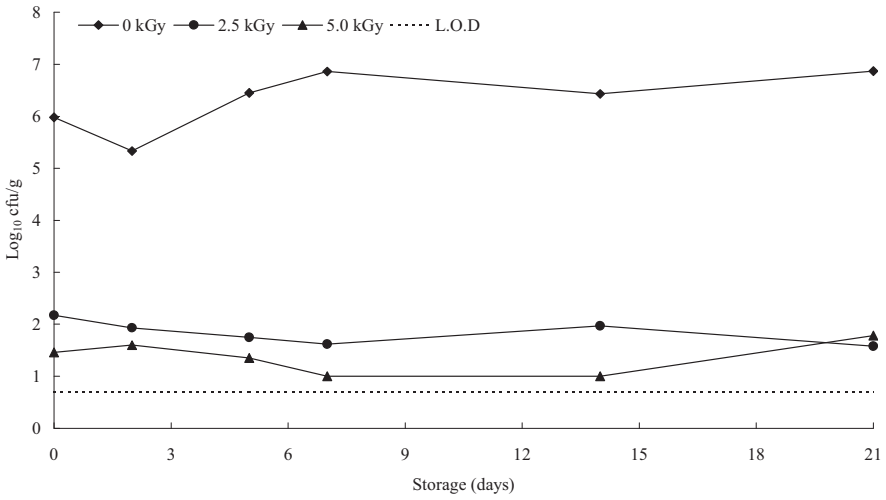

FIG. 12. Effects of irradiation and storage on the lactic acid bacteria in vacuum packed minced beef patties stored at 3 ±1°C. LOD = limit of detection (0.70 ln CFU/g).

3.3. Effect of ionizing radiation and storage on the oxidative rancidity of minced beef patties containing antioxidants at different concentrations

3.3.1. Experimental protocol 1

Data from this experiment showed that inclusion of antioxidants into the patties significantly ($p < 0.001$) reduced oxidation of the product (Figs 13–15) with TBA values being half of those in control samples without antioxidant added. Resveratrol was found to be the most effective antioxidant (Fig. 14) with the average TBA values of samples containing it being almost three times lower than those for control samples. Quercetin and epicatechin produced similar effects (Fig. 16) as oxidation levels were significantly lower than those of the controls.

The least effective antioxidant was α-tocopherol, as the TBA values of samples treated with ionizing radiation were not significantly reduced compared with those of control samples and were significantly higher than the other antioxidants tested (Fig. 14).

It was found that there was a significant difference ($p < 0.001$) in TBA values between the two concentrations of antioxidant used with lower values being obtained at the higher concentration of 550 μmol/kg compared with 110 μmol/kg. This effect was most noticeable in the irradiated samples, with little effect being observed in the non-irradiated meat patties (Fig. 15).

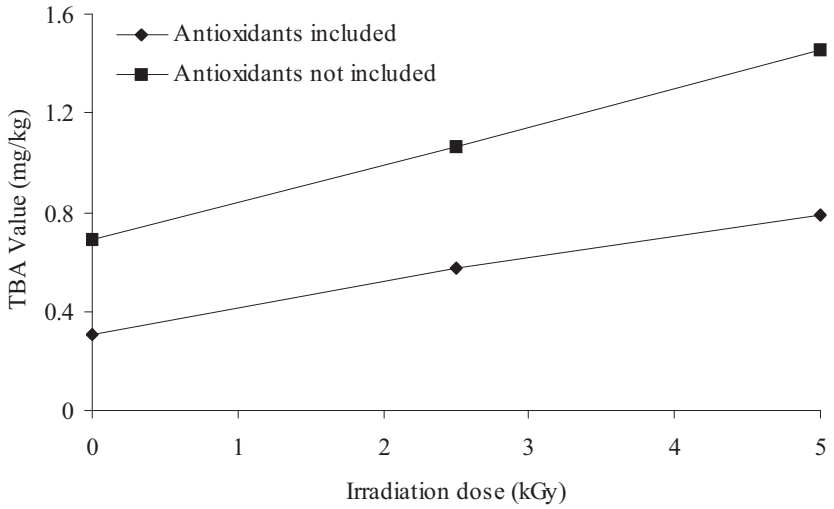

FIG. 13. Effect of irradiation treatment on the TBA values (mg malonaldehyde/kg) of minced beef patties with and without antioxidants included (for antioxidants not included SEM = 0.0595, n = 15; for antioxidants included SEM = 0.021, n = 120).

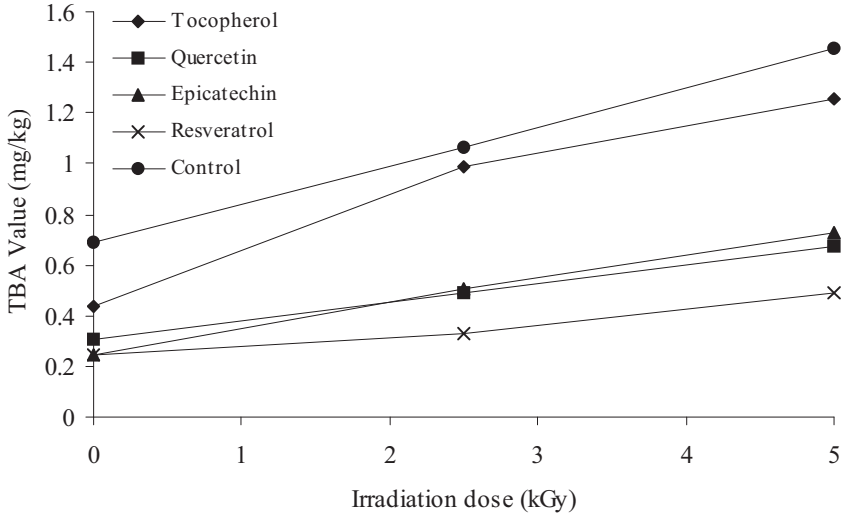

FIG. 14. Effect of including antioxidants on the TBA values (mg malonaldehyde/kg) of irradiated and non-irradiated minced beef patties with and without antioxidants included (for antioxidants not included SEM = 0.0595, n = 15; for antioxidants included SEM = 0.0421, n = 30).

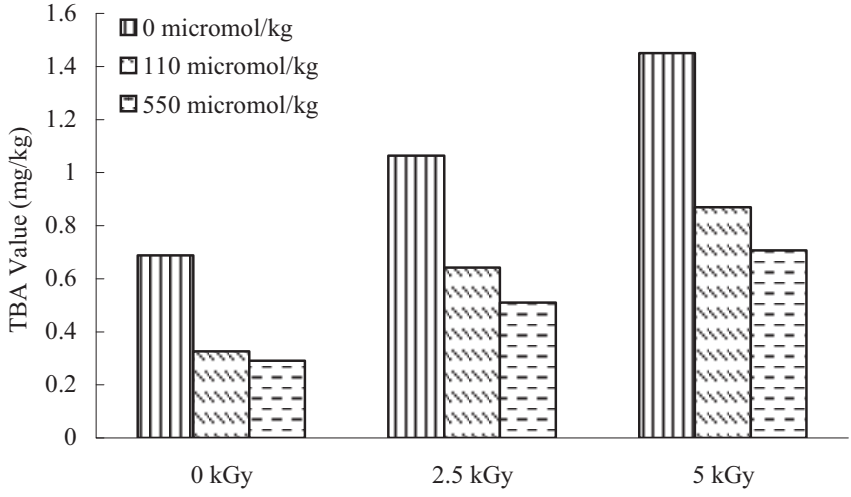

FIG. 15. Effect of including antioxidants at different concentrations on the TBA values (mg malonaldehyde/kg) of irradiated and non-irradiated minced beef patties with and without antioxidants included (for 0 μmol/kg SEM = 0.0595, n = 15; for 110 and 550 μmol/kg SEM = 0.0298, n = 60).

As expected, TBA values increased significantly ($p < 0.001$) when samples were treated with ionizing radiation, the increase being linear over the 0–5 kGy dose range (Figs 13 and 14). It was also found that TBA values increased significantly upon storage, with values at day 28 being approximately double those on day 1 of storage (Fig. 16). Figure 16 shows that for resveratrol, quercetin and epicatechin, similar TBA values were obtained during the first 14 d of storage, after which the rate of oxidation started to increase. In the case of α-tocopherol, there was a linear increase in values from day 1 to day 28 of storage similar to that of the control samples without antioxidant added (Fig. 16).

3.3.2. Experimental protocol 2

As a result of the findings discussed above, another experiment was undertaken to study a range of concentrations of the four antioxidants to determine the most effective level for reducing the oxidation of minced beef patties. As found previously, resveratrol proved to be the most effective antioxidant with α-tocopherol being least effective (Fig. 17). The concentration of antioxidant had a significant effect ($p < 0.001$) on the TBA values with a gradual decrease in oxidation being observed over the concentration range of

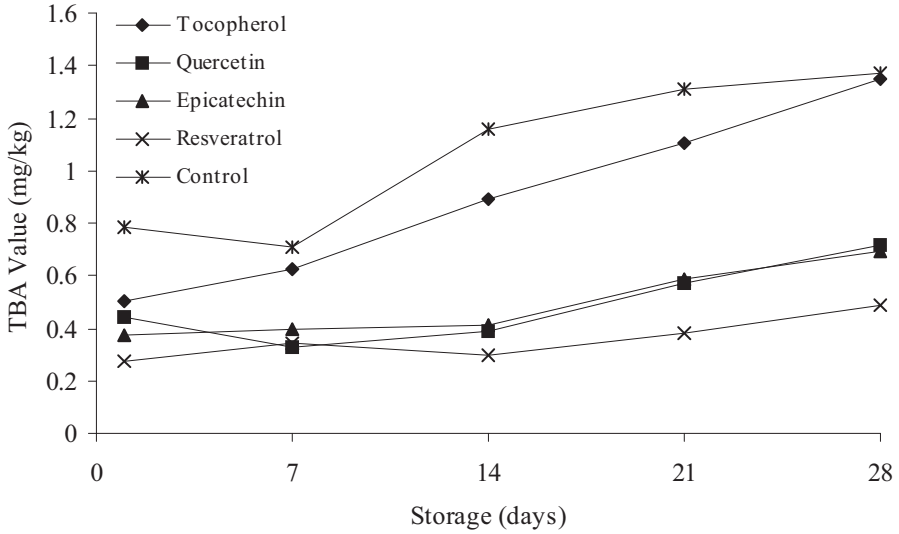

FIG. 16. Effect of storage at 3 ±1°C on the TBA values (mg malonaldehyde/kg) of minced beef patties with and without antioxidants included (control: antioxidants not included SEM = 0.0710, n = 9; antioxidants included SEM = 0.050, n = 18).

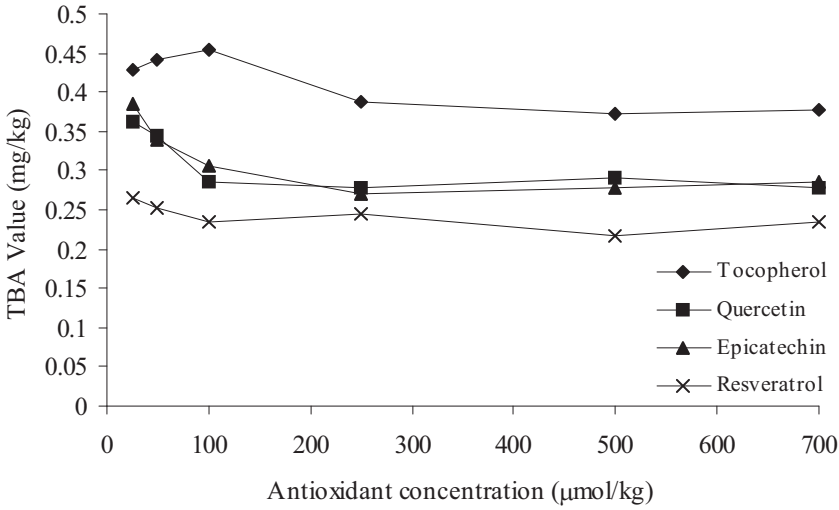

FIG. 17. Effect of different concentrations of antioxidant on the TBA values (mg malonaldehyde/kg) of minced beef patties (SEM = 0.0389, n = 12).

25–700 μmol/kg. The decrease in TBA values with increasing concentration of resveratrol did not appear to be significant as similar values were obtained at each concentration. A more noticeable decrease in oxidation values was noted for quercetin and epicatechin between levels of 25 and 100 μmol/kg, with a slight increase being observed at these concentrations for α-tocopherol, after which the values decreased. Thus, it would appear that inclusion levels as low as 25 μmol/kg would effectively reduce oxidation in meat purees, particularly if resveratrol was used as the antioxidant.

As for experiment 1, beef patties treated with ionizing radiation exhibited higher TBA values than non-irradiated samples (Fig. 18), although it was observed that there was no significant difference between values for the irradiated and non-irradiated samples containing resveratrol. Similar TBA values were measured for samples containing quercetin and epicatechin, both for irradiated and non-irradiated samples. Storage (Fig. 19) also resulted in a significant increase in TBA values.

The results obtained for the addition of antioxidants to minced beef are in agreement with studies carried out by other workers. Work by Bekhit et al. [22] on beef patties showed that resveratrol gave the highest protective effect against lipid peroxidation, with quercetin, carnosine and rutin also slowing down the process compared with the control. These workers did note that the effect of resveratrol is dependent on its concentration and method of application to meat, being more effective when applied in solution rather than

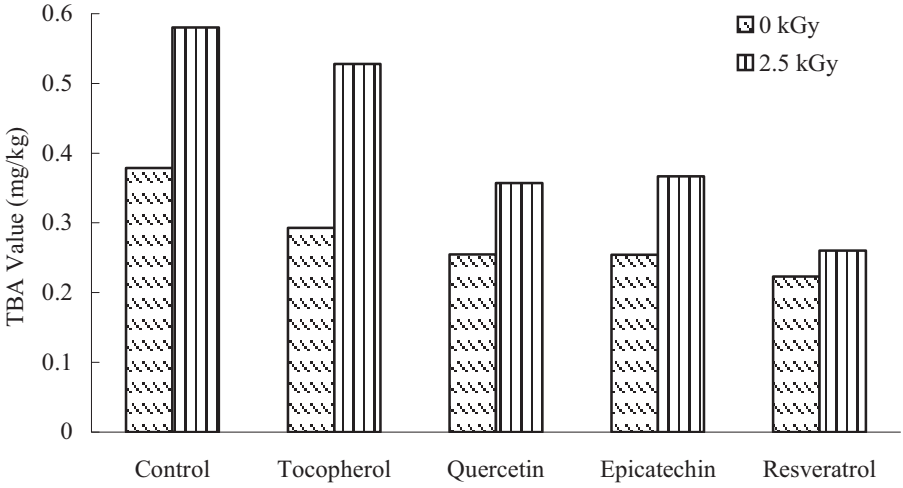

FIG. 18. Effect of ionizing radiation on the TBA values (mg malonaldehyde/kg) of minced beef patties with and without antioxidants included (control: no antioxidants included, SEM = 0.0551, n = 6; antioxidants included, SEM = 0.0225, n = 36).

in the dry form. It was found that the effect was also dependent on concentration which was also the case for the work reported here. The results obtained for α-tocopherol were somewhat surprising as this vitamin is well known for its antioxidant properties. It may be the case that as α-tocopherol is radiation

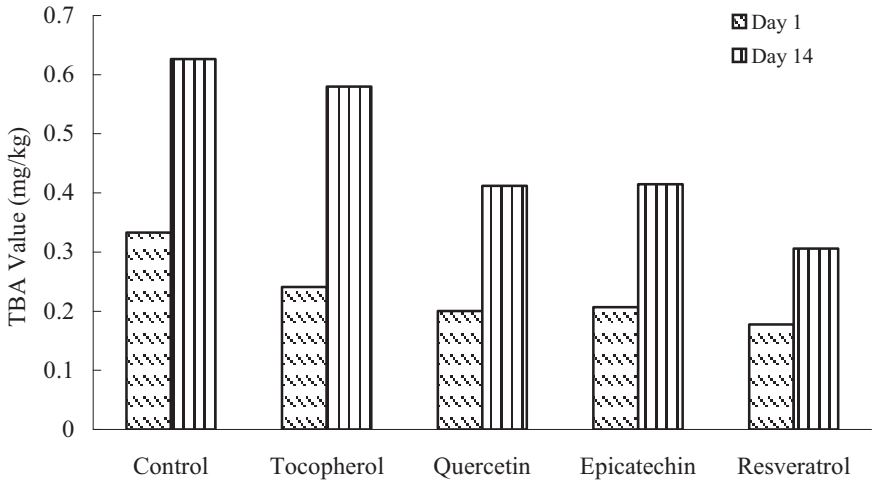

FIG. 19. Effect of storage at 3 ±1°C on the TBA values (mg malonaldehyde/kg) of minced beef patties with and without antioxidants included (control: no antioxidants included, SEM = 0.0551, n = 6; antioxidants included, SEM = 0.0225, n = 36).

sensitive, the level of the vitamin in the patties decreased upon irradiation resulting in its antioxidant activity being reduced and thus it being less effective than the other antioxidants. However, it was noted in work carried out by Ahn and Nam [23] that a combination for sesamol and tocopherol was highly effective for reducing lipid oxidation.

3.4. Effects of ionizing radiation and storage on the oxidative rancidity of salmon meat patties containing antioxidants at different concentrations

As for minced beef patties inclusion of antioxidants significantly ($p < 0.001$) reduced the levels of oxidative rancidity in the salmon patties. Data from this study showed that epicatechin was the most effective antioxidant (Fig. 20) for reducing oxidative rancidity, with TBA levels approximately 53% of those samples with no antioxidant included. This is in contrast to the results obtained for the beef patties where resveratrol proved most effective. Quercetin was the next most effective antioxidant for reducing rancidity, followed by resveratrol. As for the beef patties, α-tocopherol was the most ineffectual antioxidant, with TBA levels 5% less than those of the control samples. Work by Tang et al. [24] on the antioxidant activity of added tea catechins on lipid oxidation of raw minced red meat, poultry and fish muscle reported that tea catechins added at a level of 300 mg/kg reduced lipid oxidation, the antioxidant potential being two to fourfold greater than α-tocopherol, depending on the species.

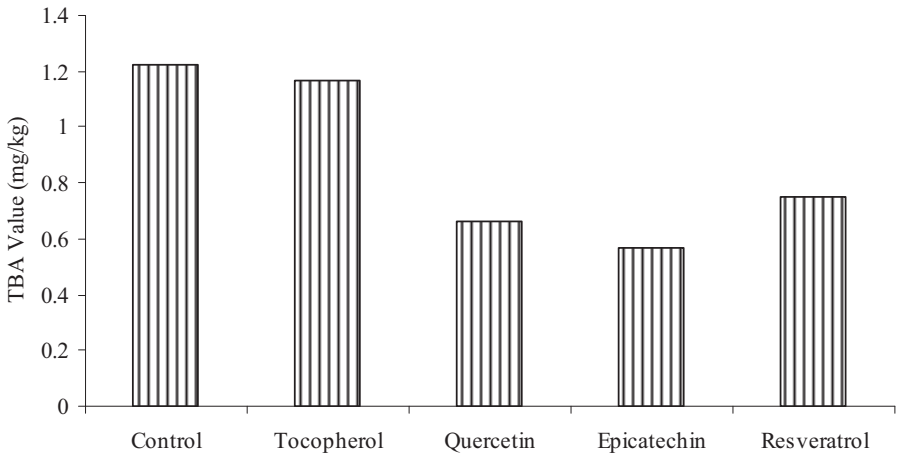

FIG. 20. Effect of including different antioxidants on the TBA values (mg malonaldehyde/kg) of salmon meat patties (SEM = 0.116, n = 12 for control, n = 72 for antioxidants).

Tang et al. [24] found that when α-tocopherol was added at a level of 300 mg/kg to minced muscle, it showed limited effects in inhibiting lipid oxidation for pig, chicken, duck and whiting. These workers suggested that chelating iron in raw minced muscle may be a possible mechanism for inhibition of lipid oxidation.

Antioxidant concentration had a significant effect ($p < 0.001$) on oxidative rancidity (Fig. 21) with overall reduction in TBA values of 31% being observed for the irradiated samples between concentrations of 25 and 700 μmol/kg. As expected, the irradiation dose significantly affected TBA levels as can be seen from Fig. 22, which also shows the effect of storage on the TBA values of the salmon purees for each of the different antioxidants and controls when irradiated or left non-irradiated. For the non-irradiated samples, similar TBA values were measured for quercetin, epicatechin and resveratrol on days 1 and 14, while the values for samples containing α-tocopherol or no antioxidant showed an increase of over 50% in both cases. A significant increase in oxidative rancidity can be clearly seen when irradiated samples were stored over the 14 d storage period. The salmon patties containing α-tocopherol showed the greatest increase in TBA values upon storage.

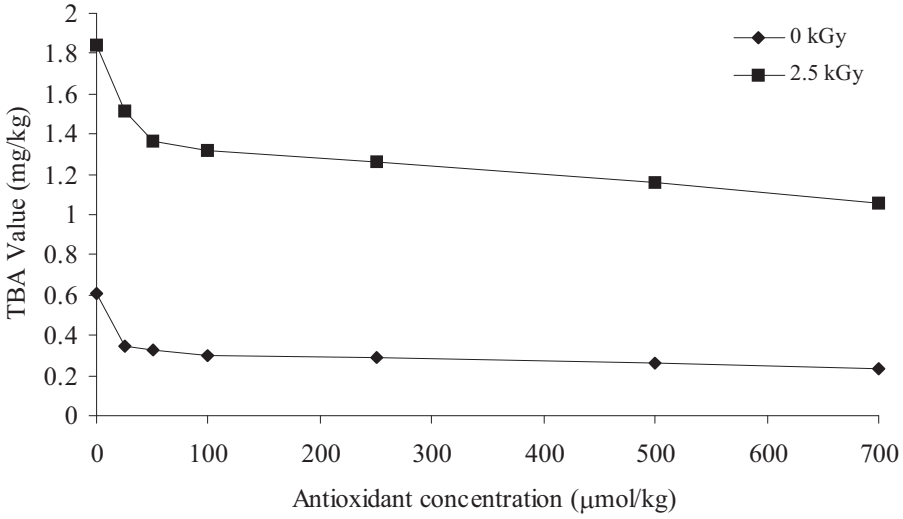

FIG. 21. Effect of concentration of antioxidant on the TBA values (mg malonaldehyde/kg) of salmon meat patties, non-irradiated and treated with 2.5 kGy (SEM = 0.088, n = 36).

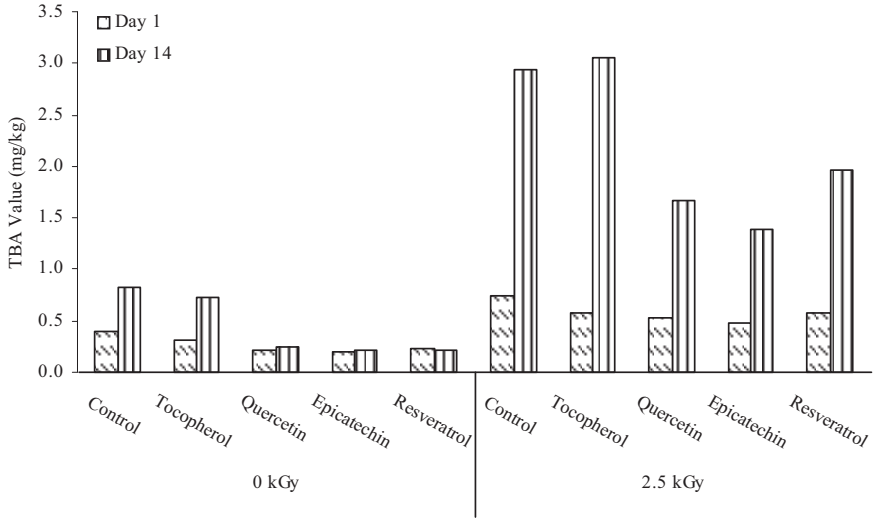

FIG. 22. Effect of storage on the TBA values (mg malonaldehyde/kg) of salmon meat patties with and without antioxidants, non-irradiated and treated with 2.5 kGy (SEM = 0.023, n = 36 for control, n = 18 for antioxidants).

4. CONCLUSIONS

The work undertaken demonstrated that:

- Irradiation doses of 2–3 kGy can extend the shelf life of ready meals and minced beef for at least 14 d at refrigeration temperatures.
- Irradiation under vacuum packaging will significantly enhance shelf life, as numbers of bacteria are significantly reduced for up to 21 d without the occurrence of unacceptable levels of oxidative rancidity.
- Storing irradiated meals or minced beef in overwrapped packages will significantly increase the microbial numbers present and result in an increase of oxidative rancidity, thereby resulting in a decrease in overall quality and acceptability.
- Irradiation will reduce the levels of vitamins in ready meals although the diminution would not have a significant impact on nutritional value.
- Addition of antioxidants to meat and fish purees prior to irradiation can significantly reduce oxidative rancidity, with the phytochemicals epicatechin, quercetin and resveratrol being more effective than α-tocopherol.

- The 2-alkylcyclobutanone (EN1785) standard method can be used to detect irradiation treatment of the meals analysed and, from previous work, has been demonstrated to be capable of detecting irradiated minced beef [25] and salmon meat [26].

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CONSUMERS' WILLINGNESS TO PAY FOR IRRADIATED PREPARED GROUND BEEF

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Abstract

This paper focuses on estimating willingness to pay for reducing the risk of contracting foodborne illness using a non-hypothetical experiment utilizing real food products (i.e. prepared ground beef), real cash and actual exchange in a market setting. Respondents were given positive information about the nature of food irradiation. Single bounded and one and one-half bounded models are developed using dichotomous choice experiments. The results indicate that individuals are willing to pay for a reduction in the risk of foodborne illness once informed about the nature of food irradiation. Respondents are willing to pay a premium of about US $0.77 for 450 g (1 pound) of irradiated ground beef, which is higher than the cost of irradiating the product.

1. INTRODUCTION

Despite the fact that food has never been as safe as it is today, food safety remains a major issue. The Centers for Disease Control and Prevention estimated that in the United States of America, 76 million people fall sick, more than 300 000 are hospitalized and 5000 people die each year from foodborne illness [1]. There were also 66 recalls for *Listeria* or *Escherichia coli* contaminated beef, pork and poultry in 2002, totalling approximately 27×10^6 kg of meat — nearly three times as much as in 2001. The largest of these recalls involved about 12×10^6 kg of food product and cost US $81 million, not including litigation costs [2]. Although the developments related to the adoption of hazard analysis critical control point (HACCP) and other safety standards may have helped reduce the incidence of foodborne illness in the USA, infections with *Salmonella*, *E. coli* O157:H7, *Campylobacter* and *Listeria*

remain and, alarmingly, continue to be an ever present phenomenon. The HACCP standard for all meat and poultry plants currently in place requires that certain standards be met throughout the industry, but it does not explicitly promote any particular antibacterial intervention and thus allows the plant some flexibility in its compliance decisions.

To really make a difference, new approaches for prevention are needed. One such approach is the use of food irradiation technology. Food irradiation is a food safety technology that can eliminate disease-causing germs from foods. As with pasteurization of milk and pressure cooking of canned foods, treating food with ionizing radiation can kill bacteria and parasites that would otherwise cause foodborne disease. Food is irradiated in a special processing facility where it is exposed to an electron beam or X ray, generated from electricity or gamma rays produced by ^{60}Co. Careful monitoring ensures that the food receives the prescribed amount of irradiation needed to destroy harmful bacteria. On the basis of decades of studies, the World Health Organization, the Food and Drug Administration (FDA), the US Department of Agriculture and the Centers for Disease Control and Prevention have endorsed the safety of irradiated food. The last organization conducted a study of the potential benefit of irradiating meat and poultry in the USA and estimated that irradiating 50% of meat and poultry will result in the prevention of nearly 900 000 cases of infection, 8500 hospitalizations and 350 deaths each year [3].

Despite these benefits, the use of this technology as an important health and food safety tool that could complement rigorous safety programmes is still limited. This paper focuses on an important component of its acceptance — consumers' willingness to pay (WTP) for irradiated food after they have been given positive information about the nature of food irradiation. The authors estimate this WTP using a non-hypothetical market experiment with real products (i.e. irradiated prepared ground beef), cash and actual exchange. A number of papers in the economics literature, including Shogren et al. [4] and List [5], have also used real products, cash and actual exchange in a market setting. Using data from their dichotomous choice experiments, the authors estimate the WTP for irradiated food using the traditional single bounded (SB) approach and a one and one-half bounded (OOHB) approach, based on Cooper et al. [6]. The authors' results suggest that individuals are willing to pay for reducing the risk of foodborne illness once informed about the nature of food irradiation with the WTP estimates varying little between the SB and OOHB estimates. Their study, however, does not provide anti-food-irradiation information to consumers, so it is possible that the results reflect the absolute maximum consumers would pay for irradiated prepared ground beef.

2. LITERATURE REVIEW

The early efforts of Bishop and Heberlein [7] found that hypothetical questions can yield responses that are similar in magnitude to those representing true market behaviour and, although there is still some debate about the method, many studies have found that elicited values can be realistic measures of WTP. Two of the main areas of concern in the WTP literature are construct validity, whether the results are consistent with economic theory, and content validity, how well the survey instrument presents the problem and captures true preferences. A source of bias that has been of particular concern in the literature is the tendency to "yea say", leading to greater estimates of WTP than is probably realistic. Evidence of this 'hypothetical bias' is widespread [8–11]. To counter such problems, some studies have investigated the means of calibrating hypothetical WTP to non-hypothetical results obtained in an experimental setting [12–15]. In general, the consistent lesson in the literature is that the more realistic the setting, the less the hypothetical bias the more valid the results will be. To the extent that real payment can be used, confidence in the results will increase [16].

A number of studies have elicited WTP for irradiated meat [17–20]. One such study, by Shogren et al. [19], is an empirical study composed of a mail survey, a laboratory experiment and a retail store semicontrolled experiment. In each case, individuals are confronted with a choice between conventional and irradiated chicken breast. They concluded that consumer choices were similar across market settings at a price premium for irradiation. Their findings also suggest that individuals are initially sceptical of irradiated food but their concerns can easily be allayed through simple educational devices. They also noted that the premium their respondents were willing to pay for irradiated chicken breasts easily covers the additional costs for commercial scale irradiation. Another study by Nayga et al. [20] 'revisited' the WTP and willingness to accept (WTA) divergence issue using irradiated ground beef and the same non-hypothetical market experiment utilized in the current paper. They found WTA/WTP ratios that are significantly lower than most such studies. Other studies have also found that consumer preferences and acceptance of the irradiation technology can be influenced by knowledge and information about the technology [17, 20–24]. For instance, Nayga et al. [24] revealed that information about the nature and benefits of food irradiation positively affects consumers' willingness to buy irradiated foods. Their study does not focus on the information effects on consumers' willingness to buy (c.f. Ref. [24]) or on the WTP–WTA divergence issue (c.f. Ref. [20]). Rather, the focus of this study is on the WTP for irradiated ground beef in Texas using data from an artefactual field experiment [25] in which respondents demonstrate their values

by exchanging money for real products. Similar elicitation approaches have been used in the past by a few studies [26, 27]. The study was conducted in Texas because no irradiated ground beef has yet been sold in Texas supermarkets and because of the relative importance of beef consumption and production in Texas. The south region that includes Texas consumes close to 40% of the total ground beef consumption in the USA [28]. Texas is also the nation's leading beef producer.

3. EXPERIMENTAL METHOD

To date, most valuation experiments have been conducted in laboratory settings and values are typically elicited using an auction mechanism [27]. In the present study, the authors conducted the WTP experiments in a more familiar setting for the consumers by carrying out the experiment in supermarkets with individual shoppers rather than in a laboratory with randomly selected groups of participants. Lusk et al. [27] argued that in store valuation has demonstrable and potential advantages for the experimenter compared with a laboratory setting, in terms of subject selection and reduced cost. Instead of using an auction elicitation mechanism, the authors conducted face-to-face WTP dichotomous choice artefactual field experiments at selected stores of the regional supermarket chain in Austin, Houston, San Antonio and Waco, Texas, from March to June 2002, using real products (i.e. ground beef), cash and actual product exchange in a supermarket setting. The sample size was determined partly as a function of the budget for the study. The study was undertaken in four cities in Texas in order to achieve a good representation of consumers in the state.

STATEMENT 1 *(Info I)*:

General statement about food irradiation excerpted from United States General Accounting Office (GAO), Washington, D.C.

"*Food irradiation* is the process of exposing food to controlled levels of ionizing radiation. Ionizing radiation is a type of energy similar to radio and television waves, microwaves, and infrared radiation. The high energy produced allows it to penetrate deeply into food, killing microorganisms without significantly raising the food's temperature.

Irradiation, within approved dosages, has been shown to destroy at least 99.9 percent of common foodborne pathogens, such as *Salmonella*

(various species), *E.coli O157:H7*, and *Listeria monocytogenes*, which are associated with meat and poultry. Irradiation can also prolong shelf life, decontaminate spices, and control insect infestation."

STATEMENT 2 *(Info II)*:

"The FDA mandates that all foods treated with ionizing energy be labeled as having been irradiated. Note that ionizing radiation has the same effect on food, but its source of energy is different. In the United States, gamma rays and electron beam are used for food irradiation.

1. Gamma rays process

The source of gamma rays is cobalt-60. The cobalt is held in thin stainless steel rods that are placed on a rack in an irradiation facility. The rack holding the cobalt containing rods is stored in a deep pool of water in the irradiation facility. When products are to be irradiated, the rack is raised from the pool and products are conveyed past the rack to absorb the gamma rays being emitted from the cobalt rods. Gamma rays are also produced from cesium-137, but those from cesium are not used for food irradiation.

2. Electron beam process

The source of electron beam is a machine, much like a television set, which utilizes electricity to generate electrons and accelerates the electrons to nearly the speed of light. When products are to be irradiated, the electron beam accelerator is switched on and products are conveyed through the stream of electrons. The electron beam process is also called electronic pasteurization."

The WTP experiments were carried out in 13 supermarkets. About 13–15 respondents per store were selected for the WTP experiments for a total sample size of 256.[1] The respondents were randomly selected as they entered the supermarket. The selected individuals were asked if they purchase and

[1] A total of 484 respondents were interviewed but only 471 of these were useful owing to incompleteness in some of the data. Of the 471 subjects, 256 were given the WTP experiments and 215 were given the WTA experiments (see Ref. [20] for results related to the WTP–WTA divergence issue).

consume ground beef and, if so, were invited to participate in a survey. If the selected individual was not eligible or refused to participate, another individual was randomly chosen. The respondents were first provided information about food irradiation as presented in statements 1 and 2. After information about food irradiation was provided, each respondent was given a pound (450 g) of non-irradiated ground beef and a randomly chosen amount of cash.[2] The respondents were told that the meat and cash were gifts for participating in the study. The respondent was then asked whether he or she would be willing to exchange the non-irradiated ground beef and the cash for the same quantity of irradiated ground beef. If the respondent accepted the bid, the cash amount was recorded as his/her WTP first bid value and the exchange was made. However, if the respondent rejected the bid, he/she was again asked his/her willingness to exchange the non-irradiated ground beef and a half value of the cash (representing second bid value for the OOHB specification) for the same quantity of irradiated ground beef. If the answer was "yes", the cash amount was recorded as his/her WTP second bid value and the exchange was made.[3]

In developing the experiment, a two day pretest was carried out in a local supermarket in College Station, Texas. The objective of the first pretest day was to use open ended WTP questions to help determine the bid values to be used in the dichotomous choice experiments. The open ended responses from these pretests were then used to select the bid values and sample sizes to be used in the dichotomous choice questions in the main experiment using the DWEABS model [29]. The DWEABS model uses an iterative procedure to select the optimal bid values as well as the sample sizes corresponding to each bid that minimize the mean square error of the welfare measure. Pretest data and total sample sizes are required as inputs for the DWEABS model to calculate optimal bid values and sample sizes corresponding to each bid. On the second day of the pretest, the dichotomous choice with follow-up experimental design was tested with the specific bid values calculated from the DWEABS model.

The experiment was designed to achieve the highest degree of realism possible. Real meat products were used in order to give the respondent an experience similar to standing at the meat counter and making choices. Real cash was used to make it clear that the respondent was making actual sacrifices

[2] Cash amounts were randomly chosen, based on values calculated from the distribution with equal area bid selection (DWEABS) model as discussed below.

[3] A reviewer expressed concerns over offering a higher incentive if the participant refused the offer the first time. The participants were not informed initially that there would be a follow-up question. However, it is possible that a participant might strategically refuse the second time, thinking there might be a third offer.

if he or she chose to pay for the irradiated meat. Although it is possible that respondents treated the gift given to them in the experiment differently than the rest of their budget, efforts were made to diminish this possibility by emphasizing that the meat and money were a gift and that they could keep these at the end of the interview. They were also told that they could stop or walk away at any time during the interview if they so desired and still keep the gift given to them. A similar method was employed by Shogren et al. [19], although in that study a payment of US $30 was given to the respondent, and they were informed that they would be offered an opportunity to purchase a good for up to US $5.

4. THE EMPIRICAL FRAMEWORK

Although statistical information could be maximized using an open ended WTP question, this ignores considerations of an individual's cognitive capacity [30]. Individuals often cannot simply state their WTP 'off the top of their heads'. The closed ended dichotomous choice format comes closest to reflecting how individuals think and what they can answer. Consequently, the authors used the dichotomous choice with follow-up experimental methodology described in this paper and estimated SB and OOHB models. The following sections discuss these models.

4.1. SB model

Assume that an individual's utility function is well behaved and defined over the commodity of interest, q, and x, a bundle of commodities other than q. The individual's optimization problem is also affected by their income, y, their characteristics, s, and the stochastic component of preference, ε. Define an indirect utility function for the individual as $v(p,q,y,s,\varepsilon)$, which implicitly assumes that optimal choices over x have already been made. Suppose the individual is confronted with the possibility of securing a change from q^0 (non-irradiated ground beef) to q^1 (irradiated ground beef) and this change costs US $\$A$. If they regard this change as an improvement, then $v(p,q^1,y-A,s,\varepsilon) \geq v(p,q^0,y,s,\varepsilon)$. The probability that an individual would accept the change at cost US $\$A$ is, therefore:

$$\Pr\{\text{response is yes}\} = \Pr\left\{v(p,q^1,y-A,s,\varepsilon) \geq v(p,q^0,y,s,\varepsilon)\right\} \qquad (1)$$

An equivalent way to express this same outcome uses the compensating variation measure, which is the quantity C that satisfies:

$$v(p,q^1,y-C,s,\varepsilon)=v(p,q^0,y,s,\varepsilon) \tag{2}$$

Thus, $C = C(p,q^0,q^1,y,s,\varepsilon)$ is the individual's maximum WTP for the change from q^0 to q^1. In general, it is assumed that the respondent knows C, but it is unknown to the analyst. The respondent will answer "no" if their maximum WTP value is smaller than the cost of the change, i.e. $C < A$. Therefore, an equivalent condition to Eq. (1) is:

$$\Pr\{\text{response is no}\}=\Pr\{C(p,q^0,q^1,y,s,\varepsilon)<A\} \tag{3}$$

The analyst treats C as a random variable, with an assumed cumulative distribution function, $G_C(\cdot)$, and probability density function, $g_C(\cdot)$. By construction, it is interpreted that $G_C(\cdot)=\Pr\{\text{response is no}\}$, and the probability of a "yes" response is:

$$\Pr\{\text{response is yes}\}=1-G_C(A) \tag{4}$$

An individual's random WTP can be formulated by assuming that C is normally distributed with $\mathrm{E}\{C\}=\mu=X\beta$, and $\mathrm{Var}(C)=\sigma^2$. Hence, $z=\dfrac{A-\mu}{\sigma}$ is a standard normal variable and Eq. (4) can be rewritten:

$$\Pr\{\text{response is yes}\}=1-\Phi(z) \tag{5}$$

where $\Phi(\cdot)$ is the standard normal distribution. Hence, the model equates to a probit model with an intercept term of $\dfrac{\mu}{\sigma}$ and a bid coefficient of $\dfrac{1}{\sigma}$:

$$\Pr\{\text{response is yes}\}=\Phi\left(\frac{\mu}{\sigma}-\frac{A}{\sigma}\right)\equiv\Phi(\alpha-\beta A) \tag{6}$$

Note that the right hand side of Eq. (1) can be rewritten as follows:

$$\Pr\{\text{response is yes}\}=\Pr\{v(p,q^1,y-A,s,\varepsilon)-v(p,q^0,y,s,\varepsilon)\geq 0\} \tag{7}$$

$$\Pr\{\text{response is yes}\}=\Pr\{\Delta v(p,q^0,q^1,y,A,s,\varepsilon)\geq 0\} \tag{8}$$

If $\Delta v(\cdot)$ equals zero, then an individual will be indifferent to the proposed improvement. From Eqs (2, 6), suppress A with C and obtain $\alpha - \beta C = 0$, where C is the expected compensating variation. Hence, given an estimate of the parameters α and β, an estimate of the individual's maximum WTP is obtained:

$$WTP = -\frac{\alpha}{\beta} \tag{9}$$

The parameters of both the SB and OOHB models are estimated using maximum likelihood. For the SB model, the log likelihood function is:

$$\ln L^s(\alpha, \beta) = \sum_{i=1}^{n} \left\{ d_i^Y \ln\left(\Phi\left(\alpha - \beta A_i\right)\right) + d_i^N \ln\left(1 - \Phi\left(\alpha - \beta A_i\right)\right) \right\} \tag{10}$$

where $d_i^Y = 1$ if the ith response is "yes" and 0 otherwise, while $d_i^N = 1$ if the ith response is "no" and 0 otherwise. The maximum likelihood estimator, denoted $\hat{\theta} \equiv (\hat{\alpha}, \hat{\beta})$, is the solution to the equation $\frac{\partial \ln L(\hat{\theta})}{\partial \theta} = 0$. The asymptotic variance–covariance matrix of $\hat{\theta}$ is given by the Cramer–Rao lower bound:

$$V\left(\hat{\theta}\right) = \left[-E\left(\frac{\partial^2 \ln L(\hat{\theta})}{\partial \theta \, \partial \theta'} \right) \right]^{-1} \tag{11}$$

4.2. OOHB model

In recent years, it has been recognized that the econometric efficiency of the SB approach is limited. Various approaches have been proposed to gather more data from each respondent and there is no consensus that one method is always to be preferred to others [31]. One approach that is now widely used is the double bounded (DB) dichotomous choice framework [32, 33]. In the DB approach, a second WTP question is asked in which the bid is increased for those who responded "yes" and decreased for those who responded "no".[4] Despite the increased econometric efficiency [32], however, Hanemann and

[4] Note that the DB approach is distinct from a bidding game specification in which the questions are repeated until a maximum WTP is identified. The bidding game approach has been rejected because its results are biased from the point at which bidding starts.

Kanninen [30] argued that there is evidence that some of the responses to the second bid are inconsistent with the responses to the first bid owing to the fact that two separate overlapping sets of bids are being requested. For example, the following scenarios can be considered: (a) the respondent answers "no" to a US $0.80 bid but answers "yes" to a US $0.40 bid, (b) the respondent answers "yes" to US $0.20 bid and answers "yes" to a US $0.40 bid. Seeing that the probability of accepting US $0.40 bid is conditioned by the first bid question, the calculated probability of accepting the US $0.40 offer from the two scenarios differs. This finding is supported by the McFadden and Leonard study [34].

A second inconsistency in the DB approach arises from what is known as the response effect. Altaf and Deshazo [35] argued that even if the respondent answers the first bid question in a neutral manner, when asked a follow-up question with a lower or a higher bid, they might not react to the second question in a neutral manner because the second question is seen as an attempt to bargain. Therefore, the response to the second bid can be biased.

Cooper et al. [6] responded to these concerns by developing an approach in which respondents are only asked a follow-up question some of the time. Their OOHB approach first involves providing a respondent with two initial values into which the true price of the good might be found. Choosing one of these two extremes at random, a first WTP question is asked. A follow-up is asked only if the respondent might rationally change their answer when asked about the second extreme. For example, a "yes" to a US $2 question might be followed by a "no" to a US $4 question. The authors show analytically that the OOHB method achieves a large portion of the efficiency gain achieved through the DB.[5]

Hence, the OOHB approach may offer most of the statistical advantages of the DB format without the problematic response effects.

The OOHB approach used here is similar to that used by Cooper et al. [6] except that a single price is first given, and then a follow-up question is asked only if a response of "no" is given to the first question. In the authors' OOHB model, therefore, there are three possible outcomes: (1) the individual responds "yes" and is given the product; (2) the individual responds "no" on the first bid but "yes" on the second, lower, offer; and (3) the individual responds "no" on both the first and second offers. Given an initial bid of US $\$A_1$, and using the same functional form assumptions as above, the probability of the

[5] Although, as noted, the anticipated efficiency gain does not materialize in their empirical application.

first event is $1 - \Phi(z_1)$, where, as above, $z_1 = \frac{\mu}{\sigma} - \frac{A_1}{\sigma} = \alpha - \beta A_1$. Those that reject the first bid are offered a second bid, $A_2 < A_1$, and the probability of accepting that bid, conditional on the fact that the first bid has already been rejected, is $g(z_1, z_2)$, where $g(\cdot)$ is the density function of $z_2 = \alpha - \beta A_2$.

The log likelihood of the OOHB model is:

$$\ln L = \sum_{i=1}^{N} \{(I_1)\log\left[\int_{-\infty}^{z_1} \Phi(z_1)dz_1\right] + (1-I_1)(I_2)\log\left[\int_{-\infty}^{z_1}\int_{z_2}^{\infty} g\left(z_1, z_2\right)dz_1 dz_2\right] + (1-I_1)(1-I_2)\log\left[\int_{-\infty}^{z_1}\int_{-\infty}^{z_2} g\left(z_1, z_2\right)dz_1 dz_2\right] \tag{12}$$

where $I_1 = 1$ if the answer to the first bid is "yes", and 0 otherwise, and $I_2 = 1$ if the answer to the second bid is "yes", and 0 otherwise. Equation (10) is treated as a bivariate function.

5. RESULTS

Table 1 presents the profile of the sample compared with the population profiles of Texas and the USA. About 57% of individuals in the sample are female, 54% are white and 45% have incomes between US $30 000 and US $75 000 per year. The sample is representative of the Texas and US populations in terms of income, employment status and marital status. Compared with the state averages, however, the sample includes more women and members of other ethnic groups (i.e. Hispanics). This was expected since the study targets shoppers in the family, who tend to be female in cities with a relatively high Hispanic population.

Table 2 exhibits the parameter estimates of the WTP models. These were estimated using Eqs (8, 10) above. The bid1 and income1 variables refer to the bid (measured in cents) and income (measured in thousands of dollars), using only the first bid values while the bid2 and income2 variables refer to the bid and income variables using follow-up bids in the OOHB model. Hence, there are no bid2 and income2 variables in the SB model because this model only uses the first bid values. Results indicate that the expected WTP equals 76.96 cents for the SB model and 76.98 cents for the OOHB model. Using Krinsky and Robb's [36] approach, the 90% confidence bounds are between 62.42 and 99.10 and between 58.90 and 153.03, respectively.

TABLE 1. RESPONDENT PROFILE COMPARED WITH TEXAS AND US POPULATION PROFILES

Variable	Survey sample	Texas[a]	USA[a]
Female	0.57	0.50	0.51
Whites	0.54	0.71	0.75
African American	0.14	0.12	0.12
Other ethnic groups	0.32	0.17	0.13
Age less than 30 years	0.17	0.46	0.42
Age between 30 and 50 years	0.50	0.30	0.30
Age greater than 50 years	0.33	0.24	0.28
Married	0.63	0.67	0.64
Employed full-time	0.55	0.59	0.60
Annual household income lower than US $30 000	0.37	0.37	0.35
Annual household income between US $30 000 and US $75 000	0.45	0.42	0.42
Annual household income greater than US $75 000	0.18	0.21	0.23

[a] Source: http://www.census.gov/main/www/cen2000.html as of 4 June 2003.

TABLE 2. ESTIMATION RESULTS

	SB		OOHB	
Variable	Coefficient	*p* value	Coefficient	*p* value
Constant1	0.7216	0.0007	0.7831	0.0001
Bid1	–0.0094	0.0002	–0.0103	0.0001
Income1	0.0001	0.9742	0.0001	0.9059
Constant2			0.9163	0.0001
Bid2			–0.0119	0.0300
Income2			0.0001	0.7285
Rho			0.9989	0.0001
Estimated WTP	76.97		76.98	
90% confidence interval[a]	62.43–99.10		58.90–153.03	
Number of observations	256		256	

[a] Calculated using Krinsky and Robb's (1986) Monte Carlo simulation technique [36]. Bids or prices used are $0.10, $0.40, $0.60, $0.80 and $1.20 for first bid values and half each of these prices for the second bid values.

It is not surprising that the OOHB results do not differ from the SB ones since both should be capturing the same underlying preferences. However, it is interesting that the efficiency gains hoped for in the OOHB approach do not materialize — the confidence interval actually increases relative to that in the SB model. Such results are not, however, without precedent. Cooper et al. [6] found very similar results in their empirical application. Their OOHB approach did not significantly change the mean predicted value, and the confidence interval actually increased.[6]

As the mean WTP values indicate, there is no statistical difference between the SB model and OOHB model WTP values. Consequently, one could conceivably use just the data from the first bids and ignore data from the follow-up questions. Herriges and Shogren [37] suggest that there are also costs associated with the use of follow-up questions. For instance, it is possible that if the individual anchors their prior WTP to the initial bid then the efficiency gains from follow-up questioning are likely to be reduced since the effective information content of the follow-up questioning is diluted by the anchoring phenomenon. The issue of anchoring was not evaluated, however, because it was beyond the scope of the current paper.

Figure 1 presents a pseudo-demand function of the predicted percentage of individuals that would accept bids from US $0 to US $2.50. The circular dots

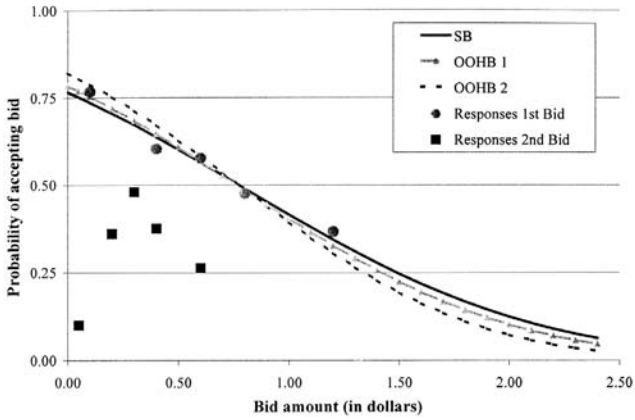

FIG. 1. Predicted probability that a bid is accepted (evaluated at an average income of US $48 175) and the actual percentage of bids accepted in the first and second rounds.

[6] It is not clear why there is a loss of precision with the OOHB approach. It is possible that this is related to the issue discussed in footnote 3. Further research on this area is warranted.

indicate the proportion of the respondents that answered affirmatively to the first bid question and the square dots indicate the responses to the second bid question. As can be seen in this case, the SB and OOHB specifications yield essentially the same WTP estimate. The main difference between the predicted acceptance rates is that the OOHB line is somewhat steeper than the SB line. This change in the slope is due to the low acceptance rates for the second bid. The steeper slopes mean that the OOHB estimate of the probability of a bid being accepted is 'tighter', which is the advantage of the OOHB specification. However, as noted above, this increase in precision is offset in this case by the greater variation in the estimated coefficients, leading to an increase in the confidence interval for the expected WTP.

Consistent with findings from other studies mentioned previously [17–19], the current findings suggest that consumers are willing to pay for irradiated food to reduce the risk of contracting foodborne illness. The USDA estimates that irradiated ground beef will cost 13–20 cents more per 450 g than non-irradiated ground beef because of the additional handling and packaging, the cost of irradiation itself, and post-irradiation testing for pathogens [2]. At these cost estimates, similar to the findings of Shogren et al. [19], the authors' WTP estimates will cover the additional costs of commercial scale irradiation. More effort is still needed though, in educating consumers about the irradiation process in order to increase their acceptance of this technology.

6. VALIDITY TESTS

Owing to the nature of WTP studies, it is important to assess the validity of the results. As noted above, the experiment was designed to achieve a high level of hypothetical validity. Construct validity is a form of validity used to assess whether hypothesized theoretical relationships between the elicited WTP and its explanatory variables are supported by the data [38]. Hence, if the results are construct valid, the estimated parameters should normally be consistent with prior expectations [39]. In the current case, two characteristics are tested: (1) is the demand curve downward sloping, and (2) whether the income effect is positive. The negative coefficients on the bid are statistically significant at the 5% level, consistent with a downward sloping demand curve. The income variable, however, is not statistically significant, suggesting that income does not have an important effect on the WTP for food safety. It is not clear, however, what feature of the authors' data or model leads to this finding.

To strengthen their analysis, the authors also tried to incorporate additional variables (i.e. demographics) to their model. Unfortunately, the econometric model did not converge when demographic variables were

included. This is not unusual. As shown by Hanemann [40], WTP is estimated on the basis of a discrete choice between two alternatives that results from a utility maximizing choice; demographic variables often have little influence on these outcomes. This condition is the analogue of the integrability conditions in conventional demand theory, which provides a criterion for determining whether a given statistical model is compatible with the economic hypothesis of utility maximization [40]. Also, it should be noted that if the indirect utility function is linear in arguments and the values a variable (e.g. demographic variable) takes for two alternatives being evaluated is constant, then the variable of potential interest essentially drops out of the comparison (i.e. it has no importance in determining an individual's choice between these two alternatives) [41]. This seriously hinders the model's capability to estimate parameters on these variables. Hence, the authors' tests of construct validity were limited to the standard economic variables of price and income.

A second type of validity test is criterion validity. Criterion validity compares WTP values either with identical markets in which the same good is bought and sold, or with an experimental market, which creates or simulates a market in which the good is actually bought or sold [39, 42]. At the time the study was conducted, irradiated ground beef was not available at any supermarket in the study locations. However, recent information about premiums of irradiated ground beef now being sold in supermarkets appear to be consistent with the authors' findings [2]; their results also appear to be consistent with findings in similar studies of WTP for food safety [17–19].

7. CONCLUDING REMARKS AND AREAS OF FUTURE RESEARCH

The realization of the benefits of food irradiation will depend on consumers' acceptance of the technology. The results of this study suggest that many individuals are willing to pay for irradiated foods once they are informed about the nature of food irradiation technology and its capability to reduce the risk of foodborne illness. Given the subject pool, the next step should be to apply the authors' methodology across a larger cross-section of the US population, both geographically and socioeconomically. In addition, the Le Chatelier principle suggests that their WTP estimates represent a measure of the upper end of the distribution of food safety preferences [43]. Hence, future research should explore other design features or alternative elicitation methods to test further the robustness of the findings. For example, future research should assess whether the results can be confirmed with experimental laboratory auction exercises or actual supermarket trials. Future research

should also further evaluate the possible effect of the 'novelty' of the product on consumers' WTP. In the current study, some subjects may have been willing to pay more for irradiated ground beef because it is new and they would like to try it. This is sometimes referred to in the literature as 'preference learning'. Preference learning or novelty effects exist if subjects are willing to pay a high premium for a good because they wanted to learn about an unfamiliar good they had not previously consumed. If this is the motive, then the authors hypothesize that the WTP will likely decline as the novelty wears off. It is also possible that some consumers have a negative WTP for irradiated foods. Very few respondents in the sample indicated a negative WTP, but future studies should not neglect this possibility. Another area of future research is assessing the effect of not only positive but also negative information (i.e., anti-irradiation information) about food irradiation on consumers' WTP, similar to the work conducted by Fox et al. [17]. Since the current study did not provide anti-food-irradiation information to consumers, it is possible that the results reflect the absolute maximum consumers would pay for irradiated ground beef.

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Annex I*

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Annex II*

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Increasing urbanization, changing socioeconomic circumstances and new working practices have all had an increasing impact on demand for prepared meals. This publication evaluates, drawing on specific examples, the value of irradiation in the preparation of ethnic meals. It discusses the efficacy of irradiation in the elimination of pathogens and the extension of shelf life without compromising overall meal quality.

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