MODIFIED ATMOSPHERE PACKAGING AND IRRADIATION PRESERVATION OF A SORGHUM PORRIDGE AND SPINACH RELISH MEAL

BY

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SUBMITTED TO THE FACULTY OF BIOLOGICAL AND AGRICULTURAL SCIENCES
UNIVERSITY OF PRETORIA
PRETORIA
REPUBLIC OF SOUTH AFRICA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
MSc (AGRIC) FOOD SCIENCE AND TECHNOLOGY
IN THE DEPARTMENT OF FOOD SCIENCE

PRETORIA
1998

I declare that the thesis herewith submitted for the MSc (Agric) degree at the University of Pretoria, has not been previously submitted by me for a degree at any other University

ACKNOWLEDGMENTS

I would like to convey my sincere gratitude and appreciation to the following people and organisations without whose help this project would not have been possible:

Prof. J R N Taylor for his constructive criticism of the thesis and his dedication and continuous help to see the project through to completion.

Dr. A. Minnaar for her continuous help, encouragement and dedication throughout the entirety of the project.

Prof. B H Bester for his support, help and suggestions pertaining to the microbiological aspects of the project.

Ingrid de Bruyn of the Atomic Energy Corporation of South Africa (Pty) Ltd. (AEC) for her contributions to the microbiological aspects of the project and for the overseeing of the irradiation of experimental samples.

Mr Don Berry (Manager – Cryogenic Applications) of BOC Gases (Pty) Ltd for providing MAP gas mixtures as well as for technical support.

Mr Dave van den Berg (Dew Crisp) and Mr Pierre Bredenhann for providing spinach.

My colleagues, Gyebi Duodu, Shone Drennan, Achim Przybyla and George Rombo for their help with the microbiological aspects of the project

Prof. & Mrs. A B Obilana, my parents, for their continued encouragement, support and prayers throughout the duration of the project.

Mrs R. Mathibe for her help in providing the recipe and cooking of the meal samples.

ABSTRACT

MODIFIED ATMOSPHERE PACKAGING AND IRRADIATION PRESERVATION OF A SORGHUM PORRIDGE AND SPINACH RELISH MEAL

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South Africa is faced with the challenge of providing food security for its entire people. Those particularly in need are people residing in the former homelands and people in the newly developed informal, urban settlements. Their food security needs include: Access to affordable, safe, nutritious foods, which meet their quality demands (e.g. in terms of culture, storability, and convenience); and the means to earn a livelihood.

Although a wide variety of traditional South African foods are prepared in the home and enjoyed by a large number of consumers, hardly any of these foods are available commercially. A typical example of one of these traditional South African foods is a sorghum porridge and spinach “morôgo” meal consumed mainly amongst the black population. This meal requires a long preparation time and has a short shelf-life.

The effects of modified atmosphere packaging, irradiation, and selected modified atmosphere-irradiation combination treatments on the microbiological stability and shelf-life of a ready-to-eat (RTE) meal consisting of spinach (morôgo) and sorghum porridge were investigated. The experiment was sub-divided into a preliminary experiment and two phases with different objectives.

The objective of the preliminary experiment was to determine the effect of a chlorine (250 mg/l) wash and blanching (77^{o}C for 6 min) in several changes of water, on the microbial count on spinach, i.e. to optimise pre-processing parameters for use in Phases 1 and 2.

Washing the spinach in chlorine led to a significant (99.92%) reduction in microbial counts. However, blanching of the spinach following the chlorine treatment did not have an effect on microbial activity. This was probably due to the fact that micro-organisms that survived the Cl_2 wash at the concentrations used were possibly resistant to blanching at the processing time and temperature (77^{o}C for 6 min) or that the number of microbes inactivated by blanching following the chlorine wash was insignificant or non-detectable by the methods used.

Blanching in more than two changes of water reduced total solids of the spinach significantly. Therefore, it was decided to use only two changes of water for the blanching treatment.

Cooking of spinach and sorghum porridge meal for Phases 1 and 2 was done according to a popular consumer recipe. The meal was dished onto a polystyrene tray, inoculated with a *Clostridium sporogenes* spore suspension, sealed in a full barrier polyethylene bag under the desired modified atmosphere conditions and irradiated using a ^{60}Co source at ambient temperature.

The objective of Phase 1 was to determine the effect of two different modified atmosphere packaging gas mixtures (MAP 1: 84.5% N_2 + 15.5 % CO_2; MAP 2: 82.3% N_2 + 15.9% CO_2 + 1.8% O_2) in combination with irradiation at five different dose levels (2, 4, 6, 8 and 10 kGy) on the inactivation of aerobic mesophilic bacteria (TPC) and *C. sporogenes* inoculated into the RTE meal, in comparison to a control (0 kGy). The purpose of this phase was to optimise the processing parameters for Phase 2.

Initially, it was found that interruptions during the irradiation processing of the RTE meal led to discrepancies in gamma D_{10}-values for *C. sporogenes* under the different MAP conditions. It was postulated that the duration of these interruptions (up to 14 h) may have been long enough for the microbes to initiate repair of the damaged DNA. After stricter control measures were taken during irradiation processing, more reliable gamma D_{10}-values were obtained. Irradiation reduced *C. sporogenes* counts and total plate counts (TPC) in the RTE meal significantly, whilst neither MAP conditions had an effect on *C. sporogenes* counts or TPC. Gamma D_{10}-values for *C. sporogenes* in the RTE meal were between 2.58 kGy and 2.60 kGy, indicating an effective inactivation rate by irradiation. A target dose of 10 kGy (actual dose 11.52 kGy) resulted in a 4 $\log_{10}$ cycle reduction in *C. sporogenes* counts. A shelf-stable meal was therefore not produced, as the irradiation dose used was not high enough to obtain a 12 D reduction in *C. sporogenes* counts.

The objective of Phase 2 was to determine the effects of the optimal combination treatment as determined in Phase 1 on the safety and shelf-life of the sorghum porridge and spinach (morôgo) RTE meal, as measured by *C. sporogenes* counts and TPC respectively.

In Phase 2, a combination of MAP 1 (84.5% N_2 + 15.5% CO_2) and irradiation at 10 kGy was used in the processing of inoculated RTE meal samples. The irradiation dose of 10 kGy was chosen for use in this phase because the two components of the RTE meal appeared to remain acceptable up to this dose level from a sensory point of view and this irradiation dose reduced inoculated *C. sporogenes* spores by approximately 4 $\log_{10}$ cycles. MAP 1 (84.5% N_2 + 15.5% CO_2) was chosen for use in Phase 2 of the research project, as it was postulated that it would minimise the effects of oxidative rancidity in the RTE meal during storage. It was also thought that these MAP conditions (84.5% N_2 + 15.5% CO_2) would inhibit the proliferation of aerobic mesophilic bacteria (TPC), thus extending shelf-life of the RTE meal during the storage period following irradiation processing. After irradiation, the samples were stored at 5^{o}C and 37^{o}C respectively during

which TPC were enumerated on days 1, 3, 5 & 7, and *C. sporogenes* counts were enumerated on days 1, 3, 5, 7, 9, 11 & 13.

Overall, MAP decreased TPC in the RTE meal when compared to the control at both 5^{o}C and 37^{o}C during the storage period. MAP also reduced the growth of *C. sporogenes* inoculated into the RTE meal at 5^{o}C beyond 5 d of storage but had no effect at 37^{o}C.

Initial *C. sporogenes* and TPC in the RTE meal were significantly reduced by irradiation compared to the control. Storage temperature ultimately determined the rate of growth of TPC and *C. sporogenes* in the RTE meal samples during the storage period. Growth of both TPC and *C. sporogenes* was faster at 37^{o}C than at 5^{o}C as this temperature is around the upper limit of the optimum growth temperature range of these mesophilic micro-organisms. MAP-irradiation combination processing was found to be synergistic with regard to TPC in the RTE meal stored at 37°C since irradiation inactivated a high percentage of the TPC and MAP kept growth of surviving TPC to a minimum.

The shelf-life of the RTE meal at 5^{o}C was as follows: 3 d for the control; 5 d for the MAP alone treatment; at least 7 d for both the irradiation alone as well as the combination treatments. At 37°C, the shelf-life of the RTE meal was: less than 1 d for both the control and the MAP alone treatments; 3 d for the irradiation alone treatment and at least 7 d for the combination treatment.

It is possible to produce a safe sorghum porridge and spinach RTE meal with a shelf-life of at least 7 d at 5^{o}C using a combination of irradiation at a target dose of 10 kGy and MAP 1 (84.5% N_2 + 15.5% CO_2) processing. However, this RTE meal is a low acid food in which *C. botulinum* can grow and produce toxins under favourable conditions. If the cold-chain is broken during distribution and/or retailing, the safety of the meal would be compromised due to the rapid growth of any surviving bacteria.

From a safety point of view, it is recommended that irradiation should not be combined with MAP 1 (84.5% N_2 + 15.5% CO_2) conditions that favour the growth of

C. sporogenes in a full barrier packaging material as it could result in the growth of the anaerobic pathogen *C. botulinum*. It is also recommended that alternative hurdles to MAP (e.g. use of nitrites and/or a_w) be used to extend the shelf-life of the RTE meal and guarantee safety.

UITTREKSEL

GEMODIFISEERDE-ATMOSFEERVERPAKKING EN BESTRALINGSPRESERVERING VAN ‘N SORGHUMPAP EN SPINASIEGEREG

DEUR

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Suid-Afrika word gekonfronteer met die uitdaging om voedselsekuriteit vir die hele bevolking te bied. Diegene wie se behoeftes spesifiek aangespreek moet word is mense wat in die vorige bedeling se tuislande sowel as in die nuut ontwikkelde informele, stedelik gebiede woon. Hul voedselsekuriteitbehoeftes sluit die volgende in: Toegang tot bekostigbare, veilige, voedsame voedsel, wat aan hul kwaliteitsvereistes (bv. in terme van kultuur, opbergbaarheid en gerief) sowel as hul behoefte om ‘n bestaan te maak voldoen.

Alhoewel ‘n wye verskeidenheid tradisionele Suid-Afrikaanse voedsels deur talle verbruikers tuis voorberei en geniet word, is min van hierdie voedsels kommersieel beskikbaar. ‘n Tipiese voorbeeld van een van hierdie tradisionele Suid-Afrikaanse voedsels wat hoofsaaklik deur swart verbruikers genuttig word, is ‘n sorghumpap en spinasie “morôgo” gereg. Hierdie gereg vereis ‘n lang voorbereidingstyd en het ‘n kort rakleeftyd.

Die effek van gemodifiseerde-atmosfeerverpakking, bestraling en geselekteerde gemodifiseerde-atmosfeerverpakkings en bestralingsbehandelings op die mikrobiologiese stabiliteit en rakleeftyd van ‘n gereed-om-te-eet (RTE) spinasie (morôgo) en sorghumpap gereg, is ondersoek. Die eksperiment was onderverdeel in ‘n loodsstudie en twee fases, elk met hul eie doelwitte.

Die doelwit van die loodsstudie was om die effek van ‘n chloorbehandeling (250 mg/l) en blansjering (77 °C vir 6 min) in verskeie veranderings van water, op die mikrobiologiese lading van spinasie te bepaal. Voorafprosesseringsparameters is dus geoptimiseer vir verdere gebruik in Fases 1 en 2.

Die wasproses van die spinasie in chloor het tot ‘n betekenisvolle (99.92%) afname in die mikrobiologiese tellings gelei. Die blansjeringsproses wat op die chloorbehandeling gevolg het, het egter geen bykomende effek gehad op die mikrobiologiese aktiwiteit in die spinasie nie. Dit was moontlik as gevolg van die feit dat mikro-organismes wat die chloorbehandeling oorleef het, bestand was teen blansjering, of dat die inaktivering van mikrobes wat oorleef het na die chloorbehandling, nie betekenisvol of meetbaar (< 10 kve/g) was volgens die metodes wat gebruik is nie.

Blansjering in meer as twee veranderings van water het gelei tot ‘n betekenisvolle verlaging in die totale vastestowwe van die spinasie. Daarom is besluit om slegs twee veranderings in water te gebruik tydens die blansjeringsbehandeling.

Die voorbereiding van die spinasie en sorghumpapgereg vir Fases 1 en 2 is volgens ‘n populêre verbruikersresep gedoen. Die maaltyd is in ‘n polistireenhouer opgedis, geïnokuleer met ‘n *Clostridium sporogenes* spoorsuspensie, verseël in ‘n ondeurlaatbare poli-etileensakkie onder geskikte gemodifiseerde-atmosfeertoestande en by kamertemperatuur bestraal deur gebruik te maak van ‘n ^{60}Co bron.

Die doelwit van Fase 1 was om die effek van twee verskillende gemodifiseerde atmosfeer verpakkings (MAP 1: 84.5% N_2 + 15.5% CO_2; MAP 2: 82.3% N_2 + 15.9% CO_2 + 1.8%

O_2) in kombinasie met bestraling by vyf verskillende dosisvlakke (2, 4, 6, 8 en 10 kGy) op die inaktivering van aerobiese mesofiliese bakterieë en *C. sporogenes* geïnokuleer in die gereed-om-te-eet gereg te vergelyk met ‘n kontrole (0 kGy, verpak in normale lug). Die doel van hierdie fase was om die prosesseringparameters vir Fase 2 te optimiseer.

Aanvanlik is gevind dat onderbrekings tydens die bestraling van die gereg gelei het tot teenstrydighede in die gamma D_{10}-waardes wat verkry is vir *C. sporogenes* onder die verskillende MAP toestande. Daar is gepostuleer dat die duur van hierdie onderbrekings (tot 14 h) lank genoeg was vir mikrobes om herstel van die beskadigde DNA te inisieer. Strenger kontrole oor die bestralingsproses het meer betroubare gamma D_{10}-waardes gelewer. Bestraling het die *C. sporogenes* en die totale plaattellings (TPT) in die gereg betekenisvol verlaag. Nie een van die MAP toestande het TPT of *C. sporogenes* tellings beïnvloed nie. Gamma D_{10}-waardes van tussen 2.58 en 2.60 kGy is verkry vir *C. sporogenes* in die gereed-om-te-eet gereg. Dit dui op ‘n effektiewe inaktiveringstempo deur die bestralingsproses. ‘n Teikendosis van 10 kGy (werklike dosis van 11.53 kGy) het tot ‘n 4 $\log_{10}$ siklus afname in die aantal *C. sporogenes* gelei. ‘n Rakstabiele gereg is dus nie geproduseer nie aangesien die bestralingsdosis nie hoog genoeg was om ‘n 12 D afname in *C. sporogenes*-getalle te bewerkstellig nie.

Die doelwit van Fase 2 was om die effek van die optimale kombinasiebehandelings, soos in Fase 1 bepaal, op die veiligheid en rakleeftyd van die sorghumpap en spinasie (morôgo) gereed-om-te-eet gereg, te bepaal in terme van *C. sporogenes* en totale plaattellings, respektiewelik.

In Fase 2 is ‘n kombinasie van MAP 1 (84.5% N_2 + 15.5% CO_2) en bestraling by 10 kGy gebruik tydens die prosessering van die geïnokuleerde gereed-om-te-eet maaltydmonsters. ‘n Bestralingsdosis van 10 kGy is gekies vir gebruik in hierdie fase aangesien die twee komponente van die gereg sensories aanvaarbaar was by hierdie dosis, asook die feit dat ‘n 4 $\log_{10}$ afname verkry is in geïnokuleerde C. sporogenes spore. MAP 1 (84.5% N_2 + 15.5% CO_2) is gekies vir gebruik in Fase 2 van die navorsingsprojek aangesien daar gepostuleer is dat die effekte van oksidatiewe galsterigheid tydens die

opberging van die gereg daardeur tot die minimum beperk sou kon word. Hierdie MAP toestande (84.5% N_2 + 15.5% CO_2) kon moontlik ook die vermeerdering van aerobiese, mesofiele bakterieë (TPT) inhibeer, waardeur die rakleeftyd van die gereg verleng kon word tydens die opbergingsperiode wat op die bestralingsproses volg. Na bestraling is die monsters opgeberg by 5°C and 37°C, respektiewelik, vir 7 d waartydens TPT en *C. sporogenes*- tellings na 1, 3, 5 en 7 dae gemeet is.

In die geheel is die TPT van die maaltyd betekenisvol verlaag deur die MAP behandeling in vergelyking met die kontrole (geen MAP behandeling nie) by beide 5°C en 37°C tydens die opbergperiode. MAP het ook die groei van geïnokuleerde *C. sporogenes* by 5 °C verminder (na 5 d van opberging). Geen effek is by 37°C opgemerk nie.

Die bestralingsproses het die aanvanklike *C. sporogenes*-getalle en TPT van die gereg betekenisvol verminder in vergelyking met die kontrole (geen bestraling nie). Die tempo van groei van TPT en *C sporogenes* in die gereg tydens opberging is uiteindelik deur die opbergtemperatuur bepaal. Groei van beide *C. sporogenes* en TPT was vinniger by 37 °C as by 5 °C gewees, aangesien 37 °C naby die boonste limiet vir optimale groei vir mesofiele mikro-organismes is.

Die rakleeftyd van die maaltyd by 5°C was soos volg vir die onderskeie behandelings:
3 d vir die kontrole; 5 d vir die MAP alleenbehandeling; ten minste 7 d vir sowel die bestralingsbehandeling op sy eie as die kombinasiebehandeling. By 37°C is die ralkeeftyd vir al die behandelings, met die uitsondering van die kombinasiebehandeling, aansienlik verkort, nl. kontrole monster sowel as die MAP alleenbehandeling het ‘n rakleeftyd van minder as 1 d gehad; die bestralingsbehandeling het ‘n rakleeftyd van 3 d gehad terwyl die kombinasiebehandeling nog steeds ten minste ‘n 7 dag rakleeftyd gehad het. Die MAP-bestralingskombinasieprosessering was sinergisties wat die TPT in die gereg opgeberg by 37°C betref het, aangesien bestraling ‘n hoë persentasie van TPT geïnaktiveer het waarna die MAP die groei van TPT wat oorleef het beperk het, tot ‘n minimum.

Dit is dus moontlik om ‘n veilige sorghum pap en spinasie gereed-om-te-eet gereg met ‘n rakleeftyd van ten minste 7 d te verkry by 5°C deur gebruik te maak van ‘n kombinasie van bestraling by ‘n teikendosis van 10 kGy en MAP (84.5% N_2 + 15.5% CO_2). Hierdie gereg is egter ‘n lae suurvoedsel (pH > 4.6) waarin *C. botulinum* onder die regte omstandighede (anaerobiese toestande asook waar ‘n verbreking van die koue ketting plaasgevind het) kan groei en toksiene produseer. Die veiligheid van die gereg sal dus in gedrang kom as gevolg van die snelle groeitempo’s van enige bakterieë wat die aanvanklike kombinasiebehandeling oorleef het.

Uit ‘n veiligheidsoogpunt word dit dus aanbeveel dat bestraling nie met MAP (84.5% N_2 + 15.5% CO_2) gekombineer moet word wat kondisies bevorder vir die groei van *C. sporogenes* in ‘n ondeurlaatbare verpakkingsmateriaal nie, aangesien dit kan lei tot die groei van die anaerobe patogeen *C. botulinum.* Dit word aanbeveel dat daar van hoër bestralingsdosisse gebruik gemaak moet word mits die maaltyd nie sensories daardeur benadeel word nie, of van alternatiewe struikelblokke (bv. die gebruik van nitriete) wat ‘n mikrobiologies veilig en stabiele maaltyd tot gevolg sal hê.

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CHAPTER 1

INTRODUCTION

South Africa is faced with the challenge of providing food security for all its people. Those particularly in need are people residing in the former homelands and people in the newly developed informal, urban settlements. Their food security needs include: Access to affordable, safe, nutritious foods, which meet their quality demands (e.g. in terms of culture, storability, and convenience); and the means to earn a livelihood.

In South Africa a wide variety of traditional African foods are prepared in the home and enjoyed by a large number of consumers. Currently, hardly any of these foods are available commercially. A typical example of one of these traditional African foods is "morôgo", a spinach or amaranth based relish consumed mainly amongst the black population, with either a maize or sorghum porridge (Mrs Rachel Mathibe, General worker, University of Pretoria, 1996, Personal communication). In a survey carried out by Van Eeden & Gericke (1996), thick maize porridge was found to be the most popular traditional cereal source, and wild greens, cooked pumpkin and a mix of spinach, cabbage and turnips were the vegetables used on a regular basis.

There are many possible problems that can be encountered with leafy vegetables (e.g. spinach), before, during and after preparation and before consumption. High perishability due to microbial and physiological activity, leads to high post-harvest losses especially in developing countries. Microbiological deterioration can also be a problem during and after preparation; and the presence of salts and anti-nutritional factors (e.g. nitrates and anti-tryptic factors) are of major concern (Urbain, 1986; Diehl, 1990; Murray, 1990).

Survival and growth of spores of pathogenic micro-organisms can be a safety concern especially as most leafy vegetables as well as cereal porridge are low acid foods (pH>4.6). The growth of some *Clostridium* species which are a major health concern with regard to food products, is a possibility under anaerobic conditions (Banwart, 1989).

The main problems and limitations associated with ready-to-eat meals include: A relatively short shelf life (e.g. maximum of 5 d at 0 to 3^{o}C including day of production and consumption - Department of Health, London, according to Patterson, Stevenson, Grant, McAteer & Stewart, 1995); concerns about microbiological safety (Wilkinson, 1990); reduced sensory quality (Zacharias, 1980) and decreased nutritive value (Bognar, 1980).

A great need exists for more novel, effective methods of food preservation. This need can be readily substantiated by documenting the grave problems, both current and future, with respect to providing an adequate diet and by drawing attention to the staggering amount of food that is unnecessarily wasted because of inadequate preservation (Fennema, 1975), especially in view of South Africa's expanding population.

Irradiation has been suggested as a method of reducing numbers of pathogens and increasing the shelf-life of certain susceptible foods (Patterson, Damoglou & Buick, 1993). From a microbiological point of view, food irradiation has two main benefits: (1) it reduces the number of pathogens and so increases food safety; and (2) it reduces the number of spoilage organisms and extends the shelf-life of the product.

Another novel technology for extending the shelf-life of RTE foods is modified atmosphere packaging (MAP). MAP may be defined as the enclosure of food products in gas-barrier materials, in which the gaseous environment has been changed (Young, Reviere & Cole, 1988) in order to inhibit spoilage agents and therefore either maintain a higher quality within a perishable food during its natural life or actually extending the shelf-life (Church & Parsons, 1995).

Farber (1991) stated that the great vulnerability of MAP foods from a safety standpoint is that since many modified atmosphere contain moderate to high levels of CO_2, the aerobic spoilage organisms, which usually warn consumers of spoilage, are inhibited while the growth of pathogens may be allowed or even stimulated. The major concern being psychrotrophic non-proteolytic clostridia, and the emergence of certain other psychrotrophic pathogens. The use of MAP in combination with one or more of the several different food preservation methods could help alleviate these problems.

The use of hurdle technology is recommended in the processing of low acid ready-to-eat (RTE) meals in order to control microbial activity in an effort to extend shelf-life (King & Bolin, 1989). Hurdle technology (also called combined methods, combined processes, combination preservation, combination techniques or barrier technology) advocates the deliberate combination of existing and novel preservation techniques in order to establish a series of preservative factors (hurdles) that any micro-organism present should not be able to overcome (Leistner 1978, 1985, 1987, 1992 & 1995, according to Leistner & Gorris, 1995). Because of their concerted, sometimes synergistic effect, the individual hurdles may be set at lower intensities than would be required if only a single hurdle were used as the preservation technique. The application of this concept has proven very successful, as an appropriate combination of hurdles achieves microbial stability and safety and also stabilises the sensory, nutritive and economic properties of a food (Leistner 1987, 1992 & 1995, according to Leistner & Gorris, 1995). The aims of combination processing technology are to enhance preservative action, reduce severity of one or all processes and to reduce cost for the quality obtained (Vas, 1981).

1.1 Objectives

The primary objective of this research was to determine the effects of combination treatments (MAP and irradiation) on the microbiological quality and shelf life of a ready-to-eat (RTE) sorghum porridge and spinach ("morôgo") meal.

The secondary objectives of this research were:

- to determine the effects of chlorine on the microbial load of spinach.
- to determine the effect of blanching in several changes of water on the microbial load of spinach.
- to determine the effect of MAP on total plate count (TPC) and *C. sporogenes* inoculated into a sorghum porridge and spinach (môrogo) ready-to eat meal.
- to determine the effect of irradiation at different doses on total plate count (TPC) and *C. sporogenes* inoculated into a sorghum porridge and spinach (môrogo) ready-to eat meal.

CHAPTER 2
LITERATURE REVIEW

2.1 Principles of food preservation

The science of food preservation is one of compromise with respect to dosage or treatment (Potter, 1986). Although most food preservation techniques are based primarily on the delay or prevention of the growth of food spoilage and poisoning micro-organisms, the preservation of quality attributes of foods is also important. With a few exceptions, all foods following processing lose quality over time. The aim of commercial food preservation is to prevent undesirable changes in the wholesomeness, nutritive value and sensory quality of food by economical methods which control the growth of micro-organisms, reduce undesirable chemical, physical and physiological changes and prevent contamination (Gould, 1989).

Preservation of food can be accomplished by chemical, biological, or physical means. Chemical preservation involves the addition to food of substances such as sugars, salt, or acids or exposure of foods to chemicals, such as smoke or fumigants. Biological preservation involves alcoholic or acidic fermentations. Physical approaches to preserving food include temporary increases in the product's energy level (heating or irradiation), controlled reduction of the product's temperature (chilling, freezing), controlled reduction in the product's water content (concentration, air dehydration, freeze-drying), and the use of protective packaging (modified and controlled atmosphere packaging and storage) (Fennema, 1975).

The control of spoilage micro-organisms is one of the major concerns of food scientists/microbiologists. This control is needed to retard or prevent spoilage and to reduce or eliminate health hazards associated with foods. The control of microbial contaminants also aids in obtaining better results when specific micro-organisms or enzymes are used in food processing (Banwart, 1989).

Four basic systems are used to aid in the control of micro-organisms in foods. These are: prevent contamination (asepsis); remove contaminants; inhibit growth of contaminants; and destroy microbial contaminants.

In most food products, two or more of these systems are used to control the microbial level. Preventing microbial contamination is practised for all foods, but since microbial contamination will still occur, other safeguards are needed (Banwart, 1989).

A problem with some of the systems in use is that they involve processes that are severe, in some cases cause major quality loss reactions. These problems could be overcome by hurdle technology i.e. combining two or more of these processes at less severe levels to control microbial proliferation (Gould, 1989).

The following review will describe various treatments, which can be given to food to aid in its preservation and how these treatments can function in combination with each other.

2.2 Pre-preparation steps

2.2.1 Washing

Most fresh produce receives some type of washing procedure after harvest. Washing is usually done by dipping the products in tanks of wash water or spraying the products as they pass underneath spray nozzles. Once washed, products either are allowed to air dry or are dried partially in centrifugal spin driers (Brackett, 1993a).

The main purpose of washing is to remove soil and debris from the product, but it also can help wash away some of the micro-organisms and thus decrease the microbial load (Brackett & Splittstoesser, according to Brackett, 1993b). The degree to which washing reduces microbial load depends on the product in question and the procedure employed (Brackett, 1993a).

The type of washing procedure itself can determine how the microflora is affected. Dipping is more likely to cross-contaminate uncontaminated products with undesirable micro-organisms from contaminated products washed previously. This possibility increases with the number of produce items that pass through a given batch of wash water. Thus, it is possible that dipping can increase the incidence of spoilage, particularly if dirty water is continually used for washing (Brackett, 1993a).

Spraying, on the other hand, causes minimal cross-contamination if wash water is recycled continually and sprayed on new products. Regardless of how the washing is done, allowing the wash water to remain on the product for an extended period of time will increase the chance for microbial growth (Brackett, 1993a).

The choice of washing equipment and other equipment used in processing vegetables depends upon the size, shape , and fragility of the particular vegetable (Potter, 1986).

Fragile vegetables such as spinach cannot be washed in agitating equipment that would break them up, but may be washed by gentle spraying on a belt (Potter, 1986). Vegetables are washed to remove not only field soil and surface micro-organisms, but also fungicides, insecticides, and other pesticides. Wash water containing detergents and other sanitizers can essentially completely remove these residues (Potter, 1986).

2.2.1.1 *Effect of washing on microbial load*

Micro-organisms rarely exist as pure cultures in nature. Foods, like the rest of the natural environment, are dynamic habitats that support the growth and reproduction of many different types of micro-organisms (Hobbs, 1986; Skoovgar, according to Brackett, 1993b). This diversity increases the chances that a spoilage micro-organism may be present on a food (Brackett, 1993a). Once located, the ability to stick to the food source and produce biofilms not only helps prevent competitive organisms from colonising the same spot, but prevents the original micro-organisms from being washed away (Brackett, 1993a).

Neelima & Splittstoesser (1990) found that both dipping and spraying reduced counts on spinach only slightly. The dominating groups of microbes included mesophiles, ranging from 10^5 to 10^6cfu/g, psychrotrophs in the range of $<10^1$ to 10^5cfu/g and lactic acid bacteria, yeasts and moulds from $<10^1$ to 10^2 cfu/g in packaged produce. The spinach seemed to contain a greater number of yellow pigmented bacteria than other produce tested, although differences in distribution may not have been significant (Neelima & Splittstoesser 1990). The observation that Gram negative rods predominate has been made on a variety of fresh cut vegetables (King, Michener, Bayene & Mihara, 1976; Manvell & Ackland, 1986; Brocklehurst, Zaman-wong & Lund, 1987).

An important issue to consider with respect to water is liquid-phase water that is in contact with vegetables as a result of condensation or washing. Such water affects the microflora in several ways. Firstly, it dissolves useable carbohydrates present or exuding from the product and serves as a growth medium. Consequently, any micro-organisms present are more likely to grow to higher populations on the product. Secondly, free water tends to raise humidity in enclosed environments such as packages, which increases the likelihood that osmotolerant organisms, most notably moulds, will grow (Brackett, 1993a).

In most cases, wash water has at least some chlorine added to it to control microbial growth. Usually the chlorine is added as sodium or potassium hypochlorite, although other forms are also used (Brackett & Splittstoesser 1992, according to Brackett, 1993a). The concentrations of chlorine normally added to wash waters can vary greatly but generally range from 5 to 250 mg/litre. However, the concentration of chlorine available to kill micro-organisms can be much less. Chlorine is very reactive and breaks down quickly in the presence of organic matter (Dychdala 1983, according to Brackett, 1993a), for example, that present in dirty water. Moreover, the effectiveness of chlorine varies with pH; it is more effective at acidic pH but less so at alkaline or neutral pH (Cord 1983, according to Brackett, 1993a). Thus, chlorine concentrations must be monitored continually to ensure that it is at the desired levels (Brackett, 1993a).

Chlorine is a potent antimicrobial agent and is particularly effective for killing microbes in suspension or on surfaces of clean equipment (Dychdala 1983, according to Brackett, 1993a). However, several researchers have found chlorine to be relatively ineffective in removing micro-organisms from fruits and vegetables. Senter, Bailey, & Cox (1987) reported that chlorine had little effect on the microflora of tomatoes, but Beuchat & Brackett (1991), found that dipping tomatoes in 200 to 250 mg/litre chlorine significantly reduced total aerobic micro-organisms but not psychrotrophs, yeasts, or moulds. However, they found no significant difference in any of the microbial groups after four or more days of storage.

Beuchat & Brackett (1990), found similar results when comparing carrots washed with and without chlorine. In this case, populations of all groups of micro-organisms studied decreased by as much as 90% immediately after being treated with chlorine. During storage however, chlorine-treated carrots developed significantly higher populations of mesophiles, psychrotrophs, yeasts and moulds than did untreated carrots. Thus, the effectiveness of using sanitizers on fresh produce will differ depending on the concentration of the sanitizer used, the product, and the amount of organic matter present (Brackett, 1993a).

Previous observations have suggested that the antimicrobial activity of hypochlorite (HOCl) solutions was related to the concentration of undissociated HOCl (Adams, Hartley & Cox, 1989). They reported that acidification of the HOCl to pH 5 increased the anti-microbial effect by a factor of 1.5 to 4.0. This observation was similar to that of Bloomfield & Miles (1979), who found that a pH decrease from 9.5 to 6.0, corresponding to a 20 fold increase in HOCl, produced only a 1.3 fold increase in activity. It was concluded that HOCl was simply the most active principle.

Since the micro-organisms that survived HOCl washing had no intrinsic resistance to HOCl, it suggest that they had not been in contact with the HOCl solution during washing (Adams, Hartley & Cox, 1989). The observation that HOCl was ineffective in preventing spoilage by bacteria closely associated with the plant tissue had been ascribed to the

inactivation of HOCl by plant tissue components before contact with the bacteria was made (Lund, 1983, according to Adams, Hartley & Cox, 1989). Such interactions were important in the creation of chlorine taints and may play a role in reducing the microcidal effect (Adams, Hartley & Cox, 1989). The coincidence of improved microbial removal with the use of the surfactant Tween, suggested that the effect was related to surface wetting (Adams, Hartley & Cox, 1989).

Mazolier (1988) according to Torriani & Massa (1994), suggested that in order to improve the microbiological quality of vegetables, the product should be treated with a chlorinated solution of 50 mg/l, whereas Strugnell (1988) according to Torriani & Massa (1994), proposed treatment in several steps: chlorination followed by neutralisation of the chlorine and then immersion in a solution of potassium sorbate.

Survival of micro-organisms in chlorinated wash water has also been attributed to attachments to a variety of other surfaces including macroinvertebrates (crustacea, nematoda, platyhelminthes and insecta) (Levy, Cheetham, Davis, Winer & Hart, 1984).

Despite the advantages of using chlorinated wash water in the food industry, it should be noted that some food processors feel that high concentrations (>200 mg/l) could cause adverse discoloration and leave off-flavours in fresh processed produce, and recommended guidelines for chlorine use were needed in the fresh produce processing industry (Hurst & Schuler, 1992).

The development of a vegetative bacterium from a bacterial spore involves loss of refractility (germination) followed by swelling and elongation of the germinated spore (outgrowth) (Wyatt and Waites, 1975). Dye and Mead (1972) reported that treatment of *C. bifermentans* with 20 μg free chlorine/ml markedly delayed outgrowth, while 50 μg free chlorine/ml allowed about 60% to germinate within 6 h but completely prevented swelling. Colony formation by spores of *C bifermentans* and *C. perfringens* which survived treatment with chlorine, were also more sensitive to heat than that of untreated spores.

Exposure to sublethal doses of hypochlorite may injure bacterial spores. Foegeding and Busta (1983a) reported injury of *C. botulinum* 62A and 12885A spores by exposure to 12 or 28 μg of free available chlorine/ml (2 min, 25^{o}C, pH 7.0). Rode and Williams (1966), found that cell walls of Gram positive bacteria and spores of *B. megaterium* were dissolved partially by hypochlorites. Protein also was removed from chlorite-treated *C. botulinum* spores (Foegeding and Busta 1983b). These results suggest that spore coats as well as underlying layers may be disrupted by chlorine, altering the permeability of spores (Foegeding, 1983). The spore coat appears to be the target of chlorine action and a barrier to chlorine permeability. Removal of coat proteins does not affect spore viability but does increase the lethal effect of hypochlorite on *Clostridium* and *Bacillus* spores (Wyatt and Waites, 1975; Bayliss, Waites and King, 1981).

2.2.2 **Blanching**

Blanching, is normally used before vegetables are further processed by freezing, drying, and canning to inactivate enzymes (Muftugil, 1985; World Health Organization (WHO), 1988). It consists of a mild heat treatment accomplished by exposing the vegetables to hot water or steam at 60^{o}C to 100^{o}C for several minutes. The treatment is acceptable since most vegetables are cooked prior to consumption (Muftugil, 1985).

Optimum blanching time was defined as the period of steam treatment sufficient to inactivate catalase and peroxidase enzymes. The optimum time of blanching varies with the vegetable (Mohammad & Ehteschamuddin, 1973).

Blanching of vegetables prior to further processing has some advantages and disadvantages. The advantages include stabilisation of texture (changes caused by enzymes), colour, flavour, and nutritional quality; the destruction of micro-organisms; and the wilting of leafy vegetables which assist in packaging. Blanching can reduce the total microbial load of raw vegetables by as much as 99.9%. The product generally has a lower microbial count after steam blanching than after hot water blanching (Banwart, 1989).

Other advantages include removal of accumulated gases and undesirable compounds (WHO, 1988). The disadvantages include loss of colour, flavour and nutritional quality; formation of cooked taste (where undesirable); some loss of soluble solids (especially in water blanching); and adverse environmental impact because of need for large amounts of water and energy (Poulsen, 1986; Williams, Lim, Chen, Pangborn & Whitaker, 1986; WHO, 1988).

2.2.3 **Cooking**

Cooking of food is such an ubiquitous and ancient practice that its role in food preservation is easily overlooked. Yet various forms of heat treatment - baking, broiling, roasting, boiling, frying, and stewing - are among the most widely used food processing techniques, in industry as well as in the home. Heat not only produces desirable changes in food, but can also lengthen safe storage times. Heating reduces the number of organisms and destroys some life threatening microbial toxins. It inactivates enzymes that contribute to spoilage, makes food more digestible, alters texture, and enhances flavour. However, heating can also produce unwanted results, including loss of nutrients and adverse changes in flavour and aroma (WHO, 1988).

Normal cooking may destroy most of the vegetative bacteria, except for thermophiles. Most of the bacterial spores will survive normal cooking procedures (Banwart, 1989). It is important that foods are cooked sufficiently to destroy certain pathogenic organisms, but they should not be heated to the extent that the nutritional value is reduced significantly. Also, the longer certain foods are heated at excessively high temperatures, the more likely is the formation of mutagens (Bjeldanes et al., 1982 according to Banwart, 1989; Lin, Lee & Huang, 1982, according to Banwart, 1989; Knize, et al., 1985 according to Banwart, 1989).

2.3 Modified atmosphere packaging (MAP)

2.3.1 Mode of action of MAP

An advanced development in packaging and refrigerated storage, is storage under modified atmosphere conditions. Modified atmosphere packaging, a technique for maintaining desirable qualities in food products, involves altering the atmosphere in the package surrounding the product. The atmosphere in the package can be altered either passively, which occurs as a result of the natural respiration of the vegetable in question, producing CO_2 as it uses up O_2; or actively, whereby the required concentrations of the different gases comprising the desired atmosphere are combined and injected into the package containing the vegetables after drawing a slight vacuum. In the case of processed or pre-cooked vegetables, no respiration is taking place. Consequently active changing of atmosphere is the method of choice (Kader, Zagory, & Kerbel, 1989; Parry, 1993).

2.3.1.1 *Gases used in the modified atmosphere packaging of food products and their effects on microbial activity*

Oxygen

Oxygen is probably the most important gas being used metabolically by both aerobic and spoilage microbes and plant tissue and taking part in some enzymatic reactions in food, but if the product is cooked, the last two will not be the case. For these reasons, oxygen is either excluded or the levels set as low as possible in MAP. The exceptions occur where oxygen is needed to avoid anaerobic conditions in food products (Parry, 1993).

Oxygen will generally stimulate the growth of aerobic bacteria and can inhibit the growth of strictly anaerobic bacteria, although there is a very wide variation in the sensitivity of anaerobes to O_2 (Farber, 1991). The exclusion of O_2 is generally desirable for the fact that it leads to the inhibition of a wide range of

microbiological spoilage agents. Its inclusion however, is essential mainly for the preservation of fresh respiring food products (Church & Parsons, 1995).

Carbon dioxide

Carbon dioxide has a powerful inhibitory effect on bacterial growth, but the actual mechanism of inhibition is still not clearly understood. It is particularly effective against Gram-negative, aerobic spoilage bacteria such as *Pseudomonas* species. However, CO_2 does not retard the growth of all types of microbes, for instance the growth of lactic acid bacteria is enhanced in conditions of high carbon dioxide and low oxygen concentrations, and carbon dioxide has little or no effect on yeast cells (Parry, 1993).

Carbon dioxide is a bacterial and fungal growth inhibitor (Wolfe, 1980; Dixon & Kell, 1989). Its mode of operation is dependent on the dissolution of the gas, which is water and fat soluble, into the packaged product (Church & Parsons, 1995). This has a number of consequences:

- the inhibitory effect is directly related to the amount of CO_2 present, i.e. the higher the CO_2 concentration, the higher the inhibitory effect. Gill & Tan (1980) found the effect of CO_2 concentration on inhibition to be linear up to 50 to 60% (of total atmosphere in terms of volume) above which there was little or no further effect on the majority of the organisms;
- the solubility of CO_2 is inversely proportional to storage temperatures and thus low temperatures have a synergistic effect upon its action (Gill & Tan, 1980);
- when dissolved, some of the gas exists as carbonic acid (Daniels, Krishnamurthi, & Rizvi, 1985) which can cause unpleasant acidic tastes (souring) when high concentrations of CO_2 are used;
- the absorption of the gas by the product causes some reduction in gas volume and consequent pack collapse. This unsightly change

in appearance is sometimes mistakenly assumed to be indicative of a sealing or material fault (Church & Parsons, 1995).

Carbon dioxide stimulates spore germination of several *Clostridium* spp. (Hambleton & Rigby 1970; Holland, Barker & Wolfe 1970). Little notice has so far been taken, however, of the fact that CO_2, at least in small concentrations, seems to stimulate the germination of *Clostridium* spores and that this may have significant influence on outgrowth and toxin formation (Enfors & Molin 1978). These authors found that carbon dioxide at 1 atmosphere stimulated the germination *of C. sporogenes* and *C. perfringens* spores, but inhibited the germination of *Bacillus cereus* spores. The stimulating effect of CO_2 was found to be higher at low pH. Foegeding & Busta (1983c) found that CO_2 stimulated the germination of three strains of *C. botulinum* spores, and germination in the absence of CO_2 was low. They concluded that CO_2 should be included in the gas atmosphere for optimal recovery of *C. botulinum*. However, substantial CO_2 levels in controlled or modified atmospheres for food preservation may prompt public health concerns. Hintalian & Hotchkiss (1986) state that CO_2 inhibits some types of micro-organisms but has no direct effect on others.

Nitrogen

Nitrogen is an inert gas, which is soluble in both water and fat. It is used in MAP primarily to displace oxygen so as to delay oxidation and prevent rancidity in foods such as nuts, or for retention of flavour in coffee beans (Parry, 1993). It can also indirectly influence the microbes in perishable foods by retarding the growth of aerobic spoilage microbes (Parry, 1993). The third role of nitrogen is to act as a filler and prevent package collapse in foods that absorb carbon dioxide (Parry, 1993).

Other gases

The potential of various other gases such as carbon monoxide, chlorine oxide, nitrogen dioxide, ozone, propylene oxide and sulphur dioxide for MAP have been

investigated experimentally but their commercial use for packaging foods is unlikely to meet with approval from the regulatory authorities (Parry, 1993).

Modified atmosphere (MA) can be introduced into a package by either gas flushing or gas compensated vacuum (Agriculture Canada, 1990, according to Powrie & Skura, 1991; O'Beirne, 1990, according to Powrie & Skura, 1991). The flushing technique involves the purging of air from the package with a continuous stream of a pre-selected gas mixture prior to sealing. In the gas compensated technique, a vacuum is created in the package to remove the air (1% or less residual) and thereafter, a pre-selected gas mixture is flushed in the package as the modified atmosphere (MA) prior to sealing. This technique involves more costly equipment and a slower production rate compared to the flushing technique (Powrie & Skura, 1991).

2.3.1.2 *Packaging systems for MAP operations*

For MAP of food products to be successful in prolonging shelf-life, suitable packaging systems are essential, particularly for adapting to abusive conditions such as temperature fluctuation and physical impacts during storage and distribution. A packaging system must be designed around the properties of the food product in question; the at-site utility; the distribution conditions; the necessity for film transparency; and gas barrier requirements (Powrie & Skura, 1991).

The major functions of a MAP packaging system include:

- protection against microbial and insect invasion into the food;
- ease of handling product at distribution, retail and consumer levels;
- resistance to physical forces such as shock, vibrations and compression impacting on food;
- regulation of gas and water vapour migration into and out of the package interior;
- ease of cooling product (Powrie & Skura, 1991).

The selection of materials for the construction of MAP packaging systems for RTE foods depend on various factors which include:

- compatibility with the food
- reliable sealability
- suitable heat transfer characteristics
- anti-fog characteristics
- physical abuse resistance
- ease of opening package
- machinability
- cost effectiveness (Kader *et al.*, 1989; Harte & Gray, 1987 and Paine, 1987, according to Powrie & Skura, 1991).

The flushing of a commodity-containing package with a specific MA gas mixture can have many rapid-response advantages such as, reducing oxidative deteriorative reactions, inhibiting microbial growth, and producing a pillow package to decrease physical damage to delicate commodity (Powrie & Skura, 1991).

An important objective of a producer of MAP food products is to properly seal packages. The seal strength is dependent on the type of film, sealer temperature, jaw pressure, thickness of plastic film and presence of foreign matter. A slight wrinkle in the seal area may be responsible for gas leakage. The simplest method to determine seal integrity is by placing the package under water and observing bubble formation with hand pressure on the package, or with vacuumisation of the test water chamber (Demorest, 1988, according to Powrie and Skura, 1991). A small amount of surfactant in the water provides easier bubble detection.

2.3.2 **Advantages and disadvantages of MAP**

The advantages of MAP according to Powrie & Skura, (1991) and Parry, (1993) are:

- Increased shelf-life allowing less frequent loading of retail display shelves;
- Reduction in retail waste;

- Improved presentation-clear view of product and all around visibility;
- Hygienic stackable pack, sealed and free from product drip and odour;
- Easy separation of sliced products;
- Little or no need for chemical preservatives (except for fresh food products);
- Increased distribution area and reduced transport costs due to less frequent - deliveries;
- Centralised packaging and portion control;
- Reduction in production and storage costs due to better utilisation of labour, space and equipment.

Disadvantages of MAP according to Powrie & Skura, (1991) and Parry, (1993) are:

- Potential of food-borne pathogens due to temperature abuse by retailers and consumers;
- Cost of gases and packaging materials;
- Cost of analytical equipment to ensure that correct gas mixtures are being used;
- Cost of quality assurance systems to prevent the distribution of leakers etc.;
- Increased pack volume which will adversely affect transport costs and retail display space;
- Capital cost of gas packaging machinery;
- Benefits of MAP are lost once pack is opened or leaks.

2.3.3 **Effect of MAP on microbial activity**

In the context of perishable foods, i.e. those subject to microbiological deterioration, modified atmosphere or vacuum packaging conditions are microbiostatic, i.e. generally capable of reducing the rate of growth of micro-organisms, and usually not microbiocidal to either aerobes or anaerobes. Further, the effect of controlled atmosphere (CA)/modified atmosphere (MA)/vacuum packaging increases as the temperature decreases (Brody, 1993).

In vacuum packaging in high-barrier materials, the air is removed to inhibit growth of aerobic micro-organisms, shrinkage, oxidation, and colour deterioration. Vacuum packaging is, in reality, a variation of CA/MA packaging because the removal of air is in itself atmospheric modification. Further, elevated levels of CO_2 and reduced levels of O_2 are produced within vacuum packages by the action of micro-organisms on the food as they consume O_2 and/or by the enzymatic respiration of food that is uninhibiting (Brody, 1993).

Atmosphere containing elevated concentrations of CO_2, coupled with high barrier packaging, are effective in inhibiting psychrotrophic organisms, particularly Gram-negative bacteria, in refrigerated products (Sander & Soo, 1978; Mitsuda, Nakajima, Mizuno & Kawaii, 1980; Hintalian & Hotchkiss, 1987; Buick & Damaglou, 1989).

The absence of O_2 permits conditions under which obligate anaerobic micro-organisms, including pathogens, might propagate and produce toxins. Further, the suppression of aerobic spoilage micro-organisms might create favourable conditions for the growth of aerobic and anaerobic pathogens (Brody, 1993).

2.4 **Food irradiation**

Food irradiation involves exposing food, either packaged or in bulk, to carefully controlled amounts of ionising radiation for a specific time to achieve certain desirable objectives (International Consultative Group on Food Irradiation (ICGFI), 1991).

Radiation is the emission and propagation of energy through space or through a material medium in the form of waves. The term radiation or radiant energy, when unqualified, usually refers to electromagnetic radiation; such radiation is commonly classified according to frequency, as radio frequency, microwave, infrared, visible (light), ultra-violet, X-rays, and gamma rays (The Van Nostrand's scientific encyclopaedia, according to Satin, 1993).

Research programmes investigating the effects of irradiation on food have been carried out under the auspices of various organisations and institutions, including the U.S. Armed Forces and the U.S. Atomic Energy Commission, the Low Temperature Research Station at Cambridge, the Torrey Research Station (fish) and the Meat Research Institute in the U.K. and the Atomic Energy Corporation (AEC) in South Africa. In spite of these massive research efforts, acceptance of the process of food irradiation by the food industry and by the general public has grown very slowly (Diehl, 1983).

Many of the practical applications of food irradiation have to do with preservation. Radiation inactivates food spoilage organisms, including bacteria, moulds and yeasts. It is effective in lengthening the shelf-life of fresh fruits and vegetables by controlling the normal biological changes associated with ripening, maturation, sprouting, and ageing. For example, radiation delays the ripening of green bananas, inhibits the sprouting of potatoes and onions, and prevents the greening of endives and white potatoes (WHO, 1988). Radiation also destroys disease-causing organisms, including parasitic worms and insect pests, that damage food in storage (WHO, 1988). As with other forms of food processing, radiation produces some useful chemical changes in food. For example, it softens legumes (beans), and thus shortens the cooking time. It also increases the yield of juice from grapes and speeds the drying time of plums (WHO, 1988). Also, as with other forms of food preservation, it has adverse effects on food quality.

One of the principal uses of irradiation is for killing the micro-organisms that cause spoilage and deterioration in food products. The amount of irradiation needed to control or eliminate these organisms depends on the radiation tolerance of the particular organism and number or “load” of such organism in the particular volume of food to be treated (WHO, 1988).

2.4.1 **Mode of action of food irradiation**

Food irradiation employs an energy form termed ionising radiation. The particular attributes of ionising radiation that make it useful for treating foods are several. Certain

kinds of ionising radiation have the ability to penetrate deep into a food. Through physical effects they interact with the atoms and molecules that make up the food and also those of food contaminants such as bacteria, moulds, yeasts, parasites and insects, causing chemical and biological consequences which can be utilised in beneficial ways. While ionising radiation frequently is referred to as high-energy radiation, the total quantity of energy needed to secure the beneficial effects with foods is relatively small, and gross changes in a food which could affect its acceptability usually do not occur (Urbain, 1986).

The interaction of ionising radiation with matter is complex. What happens depends upon the type of radiation and its energy content, the composition, physical state and temperature of the absorbing material, plus other factors such as the rate of energy deposition and atmospheric environment. The primary interaction process essentially is the transfer of energy from the incident radiation to the absorber. Only energy that is absorbed is effective in producing changes (Urbain, 1986).

There are three important aspects of the process of interaction, namely: the physical process, the chemical changes which ensue from the physical events, and the biological consequences in the cases of the target materials which include living organisms (Urbain, 1986).

The mechanisms by which micro-organisms and food constituents are altered during irradiation are initiated by direct or indirect actions (Schubert, 1974). Direct action results from direct “hits” to cells or chemical bonds causing chemical changes in the cell components of microbes (e.g. DNA), and ruptures in chemical bonds, whereas indirect effects of irradiation alters water molecules to yield highly reactive free radicals (Schubert, 1974).

When ionising radiation is absorbed in biological material, there is a possibility that it will act directly on the critical targets in the cell. The nucleic acid molecules may be ionised or excited, so as to initiate the chain of events that leads to biological change and

to cell death if the change is serious enough. This is the so-called direct effect of radiation, which is the dominant process when dry spores of spore forming micro-organisms are irradiated (Diehl, 1990).

Since the major component of cells is water, the majority of the ionising events will occur in water. The indirect effects occur as a consequence of reactive, diffusible free radicals formed from radiolysis of water reacting with DNA. The radiolysis of water results in the formation of reactive species ($OH^{\cdot}$, e_{aq}^{-}, $H^{\cdot}$, H_2 and H_2O_2). All these are reactive, but the major effective species are the hydroxyl radicals. These radicals react with organic molecules either by adding to a double bond or by extracting a hydrogen atom from a C-H bond to form water and a carbon radical (Moseley, 1989). This indirect effect of irradiation is important in vegetative cells, the cytoplasm of which contain about 80% water (Diehl, 1990), and also adversely affect food constituents. The presence of oxygen accelerates this process (Fellows, 1988).

2.4.2 **Advantages and disadvantages of food irradiation**

It has been stated that of all the efforts to minimise contamination throughout the whole chain of food production, terminal irradiation treatment of food will play the most significant role in reducing the incidence of food-borne disease (ICGFI, 1987).

According to the ICGFI (1987) the advantages of irradiation are:

- At the required absorbed dose levels, it causes negligible temperature rise in irradiated material, and it does not alter the physical state of the irradiated foods. Therefore, their fresh, frozen, or dried character is not compromised;
- Its application reduces the need for the use of some chemicals, particularly fumigants which may leave residues of toxicological significance;
- The treatment, particularly with gamma rays, involves relatively little energy consumption and is an environmentally clean process;

- It can be applied to (hermetically) packaged products which are protected against recontamination, and to some packaging materials which will not withstand a decontamination process based on heating.

The following limitations apply to the use of irradiation for decontamination of foods (ICGFI, 1987):

- High capital cost of irradiation facilities;
- Each fresh product has a threshold dose above which organoleptic changes occur.
- For most products the threshold dose will be higher when it is treated in the frozen state. This must be considered in implementing the process;
- Irradiation of certain foods will not necessarily eliminate all micro-organisms or their toxins.
- Low dose irradiation will not destroy bacterial spores, and for some products storage under appropriate temperature control is essential to prevent germination of surviving spores of *Clostridium* species.
- Although moulds and *Staphylococcus aureus* will be destroyed by irradiation, the toxins (mycotoxins and staphylococcus enterotoxins) will not. Therefore, foods prone to contamination by these organisms should be treated before toxins can be produced .

2.4.3 **Effect of food irradiation on microbial activity**

It is universally accepted that DNA in the chromosome represents the most critical target of ionising radiation (Diehl, 1990). Effects on the cytoplasmic membrane appear to play an additional role in some circumstances (Grecz, Rowley & Matsuyama 1983, according to Diehl, 1990).

Ionising radiation causes breaks in DNA strands. Restitution can occur in most of the single strand breaks, as long as the repair process is operative (Hittelman & Pollard, 1982, according to Banwart, 1989). Within the first hour of irradiation, about 90% of the DNA breaks are rejoined (Graubman & Dikomey, 1983, according to Banwart, 1989).

Most double strand breaks occur due to enzymatic incision of primary damages (Bresler, Noskin, & Suslov, 1984, according to Banwart, 1989). In general, the more complex an organism is, the more susceptible to ionising irradiation it would be.

Bacterial endospores are more resistant to the lethal action of ionising radiation than their corresponding vegetative cells by a factor of about 5 to 15 (Woese 1959, according to Moseley, 1989), and in general spores are much more resistant than vegetative bacteria regardless of the species (Bridges 1964, according to Moseley, 1989; Banwart, 1989; Diehl, 1990). However, an exception to this rule is found in the vegetative cells of *Dienococcus* species, which are more resistant than bacterial spores. Gram-positive bacteria are more resistant than Gram-negative types (Banwart, 1989). These observations are important in considering the value of ionising radiation as a means of significantly reducing the numbers of spoilage and or pathogenic bacteria in food products (Moseley, 1989).

The effect ionising irradiation has on micro-organisms depends on many factors, which include age of bacteria, suspending medium during irradiation, complexity of target organism, irradiation dose, temperature at which irradiation is carried out, and recovery assessed on complex media (Urbain, 1986).

It is well known that bacterial cells in the logarithmic phase are more sensitive to heating than those in the stationary phase. A similar phenomenon was observed for microwave irradiation (Fujikawa & Ohta, 1994) and ionising irradiation. Young cells are more sensitive to ionising irradiation than old ones with certain exceptions (Keller & Maxcy, 1984, according to Banwart, 1989; Hastings, Holzapfel, & Niemand, 1987, according to Banwart, 1989).

Although an irradiation induced chemical change may initiate an action in functioning biological systems (living organisms), the exact relationship between dose and effect is often tied to parameters of the biological system itself. For example, single cell organisms (e.g. bacteria) may be more resistant to radiation and require larger doses to

inactivate them than do more complex multicellular organisms such as insects. Some biological systems have the capability of repairing the sustained radiation damage. Such systems require larger doses to secure lethality (Urbain, 1986). Grant & Patterson (1995) found that radiation treatment is likely to be most effective against pathogenic bacteria if there is strict process control (e.g. Hazard Analysis Critical Control Point system) prior to irradiation to ensure minimum contamination with pathogenic micro-organisms; if irradiation is carried out as soon after chilling as possible; and if good dose distribution is achieved during irradiation to ensure no part of the meal is 'missed'.

Fielding, Cook & Grandison (1994) concluded that electron beam irradiation process led to a decrease in viable organisms, the decrease being proportional to the dose. Dempster, Hawrysh, Shand, Lahola-Chomiak & Corletto (1985), concluded that microbial spoilage of a perishable food product like meat could be prevented by treatment with ionising irradiation. Their results suggested that low-dose irradiation (1.5 kGy), but not less than 1.5 kGy, could improve the shelf-life of beefburgers and certain other perishable foods by at least 7 days at 3^{o}C. This extension in shelf-life is determined by the initial microbiological quality of the raw materials. According to a study carried out by Paul, Venugopal & Nair (1990), the immediate effect of gamma irradiation on lamb meat (a perishable food product) was a reduction in total bacterial count by a value of 1 to 2 log cycles/g.

2.5 Combination processing technology

Micro-organisms highly resistant to heat or irradiation pose problems in food processing because of the severe treatments required to eliminate them. Such severe treatment may adversely affect the quality of processed foods (Shamsuzzaman & Lucht, 1993). One approach for securing the desired effect (control of microbial activity) without incurring radiation damage in food is to use a smaller quantity of irradiation in combination with some other agent (a_w, high heat treatments, low heat treatments, pH, redox potential or pressure) in such a way that the two acting in concert produce the desired effect (Urbain, 1986; Gould, 1995).

2.5.1 **Mode of action of combination processing technology**

Current trends towards improvement in safety, quality and convenience of foods, and saving energy in food processing and distribution are increasing interest in developing new combination methods for food preservation (Farkas, 1990). Exploring the possibilities of using ionising radiation in combination with other preservative hurdles (environmental stress factors adversely affecting microbial growth), may significantly contribute to the development of new types of combination processes and new types of preserved foods (Farkas, 1995).

The market for pre-cooked dishes is in full expansion in developed and developing countries. According to Desmonts, Pierrat, Ingersheim, & Strasser (1995) and Gould (1995), a general trend in food preservation is towards the development of preservation techniques that are less severe and therefore less damaging to product quality while maintaining satisfactory shelf-life and safety. This could be achieved by combination processes involving heat-irradiation processing.

It is clear that a desired effect relating to the control of microbiological spoilage, insect disinfestation, decontamination of food of bacteria, yeasts, moulds and parasites, and other uses of irradiation involving living organisms always can be secured if a sufficient dose of irradiation is employed. It is also clear that often there is a limit to the amount of radiation that can be used, stemming from radiation-induced changes in foods. In some cases the dose to produce such changes is less than the dose needed to produce the desired technical effect (Urbain, 1986).

2.5.2 **Effect of combination processing on microbial activity**

The microbial safety and stability of most traditional and novel foods is based on a combination of several factors (hurdles), which should not be overcome by the micro-organisms present. This is illustrated by the so-called hurdle effect (Leistner, 1995).

For foods preserved by hurdle technology, it has been suspected for some time that different hurdles in a food could not just have an additive effect on stability, but may act synergistically (Leistner, 1995). A synergistic effect may become true if the hurdles in a food hit, at the same time, different targets (e.g. cell membrane, DNA, enzyme systems, pH, a_w, Eh) within the microbial cell, and thus disturb the homeostasis of the micro-organisms present in several respects. Therefore, employing different hurdles in the preservation of a particular food should have advantages, because microbial stability could be achieved with an intelligent combination of gentle hurdles (Leistner, 1995).

Some bacteria become more resistant (e.g. toward heat) under stress (because the synthesis of protective stress proteins is induced by heat, a_w, ethanol, etc.), or they are less heat-resistant under stress (induced by low pH or catalase inactivation in heat-injured cells) (Xavier 1994, according to Leistner, 1995). This response might influence the preservation of foods, because increased resistance under stress could turn out to be problematic in the application of hurdle technology.

Apparently, the micro-organisms in stable hurdle technology foods strain every possible repair mechanism to overcome the hostile environment. By doing this they completely use up their energy, become metabolically exhausted and die. Thus, due to autosterilization, the hurdle technology foods, which are microbiologically stable, become more safe during storage, especially at ambient temperatures (Leistner, 1995).

It is a well established fact that organisms which survive radiation treatment, like heat-damaged micro-organisms, will probably be more sensitive to environmental conditions (temperature, pH, nutrients, inhibitors, etc.) than are untreated ones (Roberts, 1970; Kiss, Rhee, Grecz, Roberts & Farkas, 1978; Farkas, 1990). However, regarding microbiological effects of combinations of heat and radiation, the time sequence of application of the combination partners may also play an important role (Farkas, 1990).

Fisher & Pflug (1977) concluded from their studies that:

- radiation and heat display a synergistic effect in the destruction of microbial spores;
- no synergism is possible unless each physiological stress is great enough to effectively destroy spores by itself;
- the synergistic mechanism has characteristics resembling each of the constituent agents, a proportional dependency on radiation dose rate, an Arrhenius dependency on temperature, and it is affected by relative humidity;
- maximum synergism occurs at those conditions where heat and irradiation are equally effective as sterilisation agents.

2.5.3 **Irradiation in combination with other food preservation methods**

In principle, any physical or chemical agent can be combined with irradiation. The combinations given below deserve special attention (Vas, 1981) as combination partners for ionising irradiation since they have been extensively reported as effective:

- Non-ionising radiations (like ultra-violet);
- Heating;
- Heat removal (refrigeration, freezing)
- Hydraulic pressure;
- Osmotic pressure

Of the chemical agents, the following combination partners for irradiation could be identified:

- Gases in the atmosphere (e.g. oxygen);
- Hydrogen ions;
- Nitrites;
- Antibiotics;
- Preservatives and other chemicals.

It is now well recognised that one of the most promising means of supplementing the effectiveness of radiation, in the control of food-borne micro-organisms without

adversely affecting organoleptic qualities of foods, is to combine it with mild heat treatment (Farkas, 1990). Heat -irradiation combination processing even has the potential to produce high quality, shelf-stable food products for use in feeding schemes for immuno-depressed individuals and for communities where facilities for frozen and/or refrigerated storage and distribution of foods are not available (Minnaar, Taylor & McGill, 1995).

There is controversy in the literature about the efficacy of combination treatments (Shamsuzzaman, 1987). Several scientists studying the effects of combination processing on micro-organisms, found synergism against bacterial spores and vegetative cells when subjected to certain combinations of heat and irradiation (Licciardello, 1964; Farkas, Incze & Zukal, 1973; Pallas & Hamdy, 1976). However, Emborg (1974) found that the radiation resistance of *B. subtilis* spores was unaffected when irradiated at 80^{o}C or 100^{o}C. Webb, Power & Ehret, (1960), according to Shamsuzzaman (1987) found no increase in heat sensitivity of pre-irradiated bacterial spores. Grecz, Upahyay, Tang & Lin, (1967), according to Shamsuzzaman (1987) reported higher radiation resistance *of C. botulinum* when irradiated at 80^{o}C to 90^{o}C than at ambient temperature. As similar phenomenon was observed in the case of *B. megaterium* (Webb et. al., 1960, according to Shamsuzzaman, 1987).

Patterson *et al*. (1995), concluded in their study that although the combination of low-dose irradiation (2 and 3 kGy) and chilled storage (2 to 3^{o}C) could produce benefits in terms of increased microbiological safety and quality of ready-to-eat meals, the effect on the sensory characteristics are complex and may limit the usefulness of the combined technologies. They also found that untrained consumers found stored irradiated meals as acceptable as the non-irradiated meals. They explained this occurrence as a result of the use of vegetables less susceptible to changes following irradiation contributing to the overall high acceptability of the meal, and also the possibility that untrained consumers may not be able to detect the differences noted by trained panellists. They concluded that a chilled, RTE meal consisting of roast beef and gravy, Yorkshire pudding, carrots, broccoli and roast potatoes could be irradiated at

2 kGy to give improved microbiological safety and extended shelf-life without significantly affecting organoleptic quality.

Improved shelf-life of meats and some other perishable products can be obtained by irradiation, particularly under vacuum. Significant shelf-life extension of MAP poultry and other perishable products are routinely obtained by industry. However the effect of the combination of MAP with irradiation appears to have received little if any attention by scientists in this area (Thayer, 1993). The combination of MAP with irradiation of pork has been investigated with promising results. It seems very probable that the combination of modern packaging techniques and ionising irradiation will increase shelf-life of meats and other perishable food products significantly (Thayer, 1993).

Farkas (1995) concluded from his study, that combinations of sensorially acceptable radiation dose and further antimicrobial stress factors may extend not only the shelf-life of specific refrigerated products, but they can improve their microbiological safety, for instance, in case of temperature abuse.

2.5.4 **Irradiation in combination with MAP**

Very little work has been done on the effect of irradiation in combination with MAP on ready-to-eat cooked meals. As a result, very little literature is available on the subject, as opposed to the combination effect on fresh pork, beef or poultry products

The amount of irradiation that can be applied to a particular food, and hence the extent of microbial kill, is limited by undesirable changes in flavour, odour, appearance and texture which may occur. The “threshold dose” above which these organoleptic changes can be detected, varies with different foods. By using low-dose irradiation in combination with other treatments, the desired microbial effect can be achieved without significant loss of organoleptic quality (Grant & Patterson, 1995). The combination of low-dose irradiation and MAP or sous vide packaging has potential as it could be possible to reduce the numbers of spoilage and pathogenic organisms by irradiation and suppress the growth of

surviving micro-organisms during storage by MAP or sous vide packaging without significantly affecting organoleptic quality (Grant & Patterson, 1995).

There have been concerns expressed that MAP alone, or in combination with low-dose irradiation, might favour the growth of, and toxin production by, *Clostridium botulinum* particularly under conditions of temperature abuse (Lambert, Smith & Dodds, 1991). The main concern is that irradiation and/or MAP may inhibit the spoilage bacteria sufficiently to allow toxin formation while the product is still organoleptically acceptable. A further concern is the possibility that irradiation in conjunction with MAP may activate spores and enhance toxin production by *C. botulinum*, if the product is temperature abused (Lebepe, Molins, Charoen, Farrar & Skowronski, 1990).

A factor that can influence the microbiocidal effectiveness of irradiation is the nature of the atmosphere in contact with target cells. Vegetative cells of some bacteria are more susceptible to the effects of irradiation in the presence of O_2 than under anaerobic conditions. Like irradiation at higher temperatures, however, irradiating in the presence of O_2 may produce off-odours and off-flavours due to lipid oxidation (Monk, Beuchat & Doyle, 1995).

Irradiation treatments are designed to reduce or eliminate viable micro-organisms that may be present in foods. However, if some cells do survive, they may require optimum environmental conditions to enable repair of sublethal damage, just as do heat-damaged cells (Rowley & Brynjolfsson, 1980, according to Monk, et. al. 1995). For this reason, the combined effect of treatment with irradiation in conjunction with other modes of preservation can be used to achieve low population of viable cells initially and control growth during storage (Monk, et. al. 1995).

Elimination of oxygen in vacuum-, nitrogen- or carbon dioxide-packed (MAP) foods predictably alters the spoilage flora by preventing the growth of strict aerobes unless some alternative electron acceptor is available to them. In addition, restriction of oxygen lowers the growth rates and yields of many facultative anaerobes and reduces the amount

of energy available to them from fermentative, compared with oxidative, metabolism. Consequently, oxygen-free packaging is particularly useful in combination with other preservation procedures that place extra energy demands on the cell, for instance reduction in pH value, addition of weak organic acid preservatives, reduction in a_w, slight heat treatment and irradiation (Gould, & Jones, 1989).

No research work has been done involving MAP-irradiation combination processing to preserve traditional South African foods or meals. Knowledge of the two processing parameters on the microbiological safety and stability under various storage conditions is required prior to commercialisation of these foods or meals.

CHAPTER 3
EXPERIMENTAL

3.1 Experimental design

To determine the optimum combination parameters for MAP-irradiation processing of RTE sorghum porridge/spinach (morôgo) meal, the research work was sub-divided into a preliminary experiment and two phases with different objectives.

3.1.1 Preliminary experiments

The objectives of these preliminary experiments were to determine the effect of chlorine (prepared by diluting ACE bleach (Pick 'n' Pay supermarket) with an NaOCl concentration of 3.5% (m/v) to a concentration of 250 mg/l available Cl_2) and blanching in several changes of water at 77^{o}C for 6 min on TPC and spore count in the spinach, i.e. to optimise pre-processing parameters for use in Phases 1 and 2. The concentration of chlorine solution was chosen with the view to inactivate as many micro-organisms as possible from the spinach before the preparation of the meal.

3.1.2 Phase 1

The objective of Phase 1 was to determine the effects of a combination of MAP and irradiation on the survival of the spores of *C. sporogenes* and subsequent growth of the vegetative cells in order to determine the optimal treatment combination which would give the most significant advantage with regards to maximum reduction of microbial load in the packaged product (porridge and "morôgo"), hence extending its shelf-life. The sorghum porridge and spinach components of the meal were put in a polystyrene tray and each component was then inoculated (using a Gilson pipet with sterile 1 ml tips) on the surface with 1 ml of a suspension of *C. sporogenes* spores (10^7 to 10^8 spores /ml). The inoculated RTE meal samples were then subjected to the following treatments:

- MAP 1 (84.5% N_2 + 15.5% CO_2)
- MAP 2 (82.3% N_2, 15.9% CO_2 + 1.8% O_2)
- Irradiation at 0, 2, 4, 6, 8 & 10 kGy (carried out at ambient temperatures)
- Combination of MAP and irradiation treatments

The experiment was carried out in triplicate. The MAP gases used were recommended by Don Berry, Manager Cryogenic Applications (1996 - Personal Communication). The irradiation dose levels of up to 10 kGy were chosen as this was the highest dose allowed at the time by the Codex Alimentarius Commission for the processing of foods. Since then a press statement was issued by the Joint WHO/FAO and International Atomic Energy Agency (WHO, 1997) that "strictly from the scientific point of view, no ceiling should be set for food irradiated with doses greater than the currently recommended upper level of 10 kGy."

3.1.3 **Phase 2**

The objective of Phase 2 was to determine the effects of MAP/irradiation combination on the shelf-life and safety of the processed spinach ("morôgo") and sorghum porridge ready-to-eat meal after a storage period of 7 d at both 5°C and 37°C. MAP 1 (84.5% N_2 + 15.5% CO_2) and irradiation at 10 kGy were determined from the previous experiments as being the most effective in reducing TPC and *C. sporogenes* counts and were therefore used in this experiment. The two temperatures (5°C and 37°C) were chosen in order to simulate proper cold chain storage conditions and severe temperature abuse conditions, respectively. The experiment was also carried out in triplicate and the different treatments were as follows:

<u>Storage at 5°C</u>

Control:	No irradiation (0 kGy), No MAP (MAP 0)
MAP:	0 kGy, MAP 1 (84.5% N_2 + 15.5% CO_2)
Irradiation:	10 kGy, MAP 0
Irradiation + MAP:	10 kGy, MAP 1 (84.5% N_2 + 15.5% CO_2)

Storage at 37°C

Control:	0 kGy, MAP 0
MAP:	0 kGy, MAP 1 (84.5% N_2 + 15.5% CO_2)
Irradiation:	10 kGy, MAP 0
Irradiation + MAP:	10 kGy, MAP 1 (84.5% N_2 + 15.5% CO_2)

Irradiation processing were carried out at ambient temperatures (20 to 25°C). During storage at 5°C and 37°C total plate counts were enumerated on days 1, 3, 5 & 7and spore counts were enumerated on days 1, 3, 5, 7, 9, 11 & 13 as described in section 3.5 to 3.8.

3.2 Determination of total solids

Moisture content and total solids were determined using an oven drying method as described by Nielsen (1994). Moisture dishes were dried at 103°C for 1 h, and cooled in a desiccator for 10 min. The dishes were weighed and their weights recorded. Approximately 5 g of finely ground sample was weighed into the dishes and dried at 103°C for 3 h. The dishes with the samples in them were then cooled in a desiccator for 15 min weighed and the weight recorded. The total solids was calculated using the following formula:

% Total Solids = weight of sample after drying / weight of sample before drying x 100

3.3. Preparation of RTE meal

Sorghum porridge and spinach (*Spinacia oleracea*) relish were prepared as recommended by Mrs Rachel Mathibe (General worker, Department of Food Science, University of Pretoria, 1996 - Personal communication).

Young, soft tender leaves of spinach for the preliminary experiments were obtained from Dew Crisp (a vegetable processing company in Johannesburg) and those for Phases 1 and 2 from the Johannesburg fresh produce market. The shoots were rinsed, the leaves picked off the shoots and chopped. The chopped spinach leaves were then rinsed in chlorinated

water (250 mg/l) to reduce microbial load. The washed leaves were transferred to a water heat exchanger (kettle), and blanched in two changes of hot water at approximately 77^{o}C for 6 min as recommended by Luh & Kean (1975). This step was required to help reduce the amount of various anti-nutritional factors (e.g. nitrates and oxalates) present in the spinach leaves (Duodu, 1998), and to inactivate enzymes that could lead to physiological deterioration.

For preparation of the "morôgo", washed/blanched spinach leaves (100 g) were added to 50 ml of water, and 0.6 g of salt added. The mixture was cooked (stirring occasionally) until the water had totally evaporated. In a clean pan, 60 g of Farmgirl tomato and onion mix (Pick-n-Pay supermarket, Pretoria) was simmered for 10 min. White pepper (0.60 g) was added for taste. The cooked spinach was then added and the mixture cooked for a further 25 to 30 min.

For the sorghum porridge, Super Mabela sorghum meal from Nola (Randfontein) was used. Water (100 ml) was brought to a boil in a clean saucepan. Sorghum meal (45 g) was mixed with 20 ml of cold water to form a paste, the paste was then poured into the boiling water and the mixture continuously stirred to prevent formation of lumps. The saucepan was covered with its lid and maintained at the same (boiling) temperature until a thick paste was formed (approximately 25 to 30 min).

The porridge and the "morôgo" were left to cool to ambient temperature (20 to 25^{o}C), then dished out into polystyrene trays (obtained from Pack'n' Spice, Pretoria) and inoculated on the surface (using a Gilson pipet with sterile 1 ml tips) with *C. sporogenes* spore suspension, as described in 3.1.2. The meal was packaged in full barrier polyethylene bags [polyvinylchloride-coated polyester; 15 μm barrier abuse layer laminated with 50 μm linear low density polyethylene, (Cryovac Pty. Ltd., Kempton Park)] and sealed with the desired atmosphere (BOC Gases, Germiston) in a benchtop Vacpac machine (B.T. Enterprises, Johannesburg). The packaged meal samples were stored at 5^{o}C over night, and transported in cooler boxes to the Atomic Energy Corporation (AEC) irradiation facility at Pelindaba, RSA, where they were exposed to a

^{60}Co source (at ambient temperature – 20 to 25°C) until the desired dose was reached. Samples (for treatments requiring 8 and 10 kGy irradiation target doses) were irradiated to 6 kGy (target dose) on day 1 and left overnight at ambient temperatures (20 to 25^{o}C) before being irradiated to 8 and 10 kGy (target dose) the following morning. During Phase 1, samples (at 20 to 25^{o}C) were analysed as soon as possible after irradiation. During Phase 2, samples for day 1 were analysed as soon as possible after irradiation (0 to 2 hr), whilst the remaining samples were stored at 5^{o}C and 37^{o}C until analysed. Figure 2 shows the freshly prepared RTE meal on a polystyrene tray with and without MAP prior to irradiation processing.

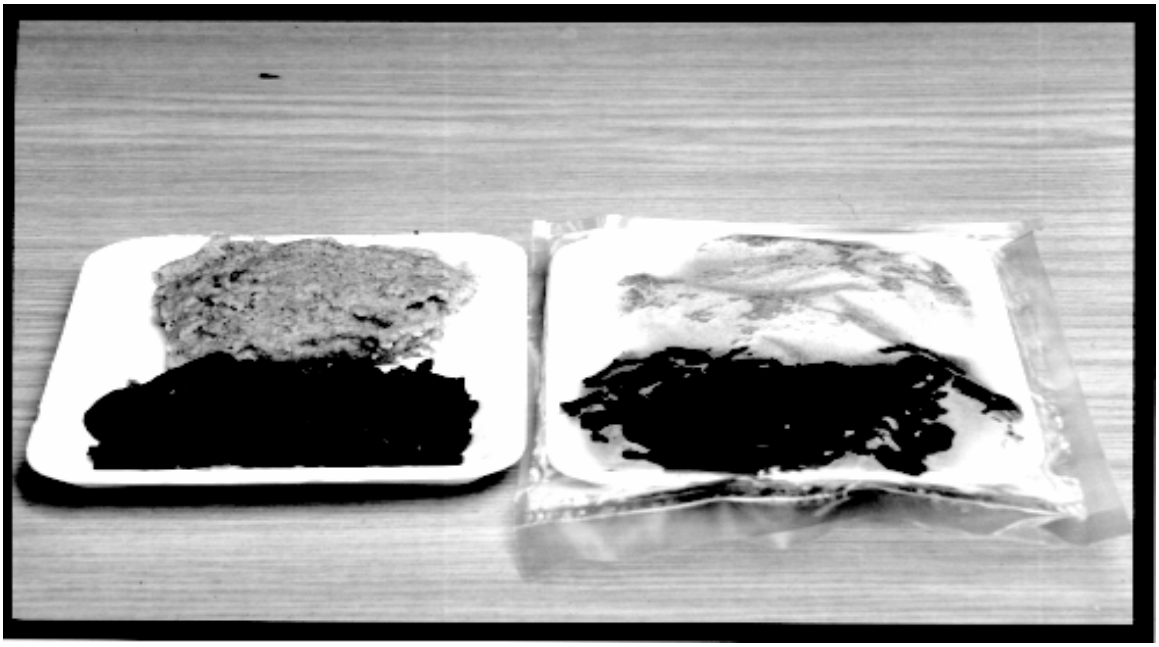

Figure 1. Freshly prepared RTE sorghum porridge and spinach meal with (right) and without (left) MAP

3.4 Preparation of spore suspension for inoculated pack studies

Spores of *C. sporogenes* were produced by the biphasic method of Anellis, Berkowitz, Kemper & Rowley (1972). The spores of *Clostridium sporogenes* were propagated in broth composed of 5% (m/v) Tryptone (Oxoid), 0.5% Peptone (Biolab), and 0.125% K_2HPO_4 (Merck), and adjusted to pH 7.5 with 5 M KOH before autoclaving. Filter

sterilised $NaHCO_3$ was added to a final concentration of 0.075% prior to inoculation. The inoculation sequence is shown in Table 1. For sporulation, the agar phase was of the same composition, except that the $NaHCO_3$ was omitted and 0.1 % yeast extract (Merck) and 3 % agar were added. The agar phase (1000 ml) was prepared on the day of use in 2800 ml Fernbach flasks, autoclaved, cooled rapidly to 20 to 30°C, and overlaid aseptically with a 2 % $(NH_4)_2SO_4$ sterile liquid phases and then inoculated. Incubation was at 30°C for 5 to 6 d. The spores were harvested, suspended in 0.067 M Sorenson phosphate buffer (pH 7.00) and washed by three successive centrifugations at 2500 r/min for 20 min at 2 to 5°C, each time re-suspending the spores in the phosphate buffer, and then stored in 100 ml of buffer at 2 to 5°C until used.

Table 1 Inoculation sequence of *C. sporogenes* for the preparation of spores by the biphasic method (Anellis *et al*., 1972)

Inoculation sequence	Incubation at 30°C (h)
Spore stock[1] (5 ml)	
|	
20 ml broth[2] (5 ml)	24
|	
20 ml broth[2] (5 ml)	4
|	
20 ml broth[2] (5 ml)	4
|	
Biphasic system[2]	5-6 d

[1] Brain liver heart (Difco); heated at 80°C for 10 min and cooled rapidly to 20 to 30°C.
[2] See section 3.4

3.5 Sampling and preparation of homogenate for microbiological assay

The RTE meal samples were aseptically sampled by weighing 22 g well-mixed sample into a sterile stomacher bag, and then stored at 0 to 4.4^{o}C until analysed. To each bag 198 ml of sterile peptone water diluent were added and then placed in the stomacher (supplied by Art Medical Equipment, Johannesburg) and the contents macerated for 0.5 to 1 min, depending on the sample. Decimal dilutions were prepared in 9 ml diluent. All dilutions were vigorously shaken 25 times in a 30-cm arc within 7 s (Speck, 1984).

3.6 Total plate count (TPC) method

The spread plate method was used for the enumeration of total plate counts. Onto dried tryptic soy agar (TSA) plates 0.1ml of the relevant dilution was placed. The sample was immediately spread over the surface of the plate using a sterile bent glass rod ('hockey stick'). The plates were dried for 15 min, inverted and incubated at 35 ± 2 ^{o}C for 48 ± 3 h. Each sample was plated in triplicate i.e. three plates at each dilution. After incubation, the colonies on triplicate plates were counted and results recorded as colony forming units (cfu/g) (Speck, 1984).

3.7 Total spore count (TSC) method

Mesophilic aerobic spores were also enumerated, as follows. Ten millilitre, 1 ml and 0.1 ml of the homogenate were pipetted into three flasks containing 100 ml of melted sterilised tryptose glucose extract agar (Merck) at 45^{o}C, and mixed thoroughly. The flasks were transferred immediately to a water bath at 80^{o}C and held for 30 min after reaching 80^{o}C. The flasks were then cooled in cold tap water making sure the agar did not cool to below 45^{o}C. They were then transferred to a 45^{o}C waterbath for no more than 10 min. Each 100 ml inoculated medium was pour-plated into a set of five plates (about 20 ml/plate). When the agar had solidified, the plates were inverted and incubated at 35 ±

2°C for 48 ± 2 h. The sum of the counts on the five plates represented the number of aerobic mesophilic spores/g for the 10 ml sample (counts from plates with 1 and 0.1 ml were multiplied by 10 and 100, respectively to give the number of spores /g). A range of 20 to 150000 spores/g could be detected by this method (Speck, 1984).

3.8 **Enumeration of *C. sporogenes***

The spores of *C. sporogenes* were enumerated by a modified version of the methods described by Anellis, Shattuck, Rowley, Ross, Whaley & Dowell, (1975). Cells were not heat shocked and Peptone P (Biolab) was used instead of Thiotone (BBL).

Culture media and reagents used were:

- Tryptic Yeast Thioglycolate agar [5.0% peptone P (Biolab), 0.5% yeast extract (Biolab), 0.5% tripticase (Biolab), 0.05% sodium thioglycolate (Merck), and 0.75% agar (Biolab)]
- 0.75% $NaHCO_3$ (0.25ml filter-sterilised)
- NaOH (5M) to adjust pH of media to 7.2
- Long narrow tubes (11 by 202 mm) used to ensure an anaerobic environment in the media without additional measures such as an anaerobic glove cabinet or anaerobic flasks.

Enumeration:

Aliquots (1 ml) of the homogenate were inoculated into each of triplicate tubes (11 by 202 mm) containing 0.25 ml of filter-sterilised 0.75% $NaHCO_3$, and 10 ml sterilised molten agar medium at 45°C was then added to the tubes. Pouring of the agar into the tubes resulted in a swirling motion, which produced uniform mixing of the spores with the tube contents. The contents of the tubes were then covered with 1.5 to 2 cm of the same medium. After incubation at 35 ± 2°C for 48 ± 2 h, the mean of the number of colonies formed in the triplicate tubes was calculated.

3.9 Statistical analysis of data obtained from microbiological tests

The data were analysed using Statsgraphic (Statistical Graphic System) version 5.0 from the Statistical Graphics Corporation and Statistica version 4.00.950 from the Microsoft Corporation. Analysis of variance was used to evaluate the data and evaluations were based on a 5% significance level. When the F-test was significant, differences between treatments were determined using least significant difference (LSD) values. Regression analysis was performed on the data from Phase 1 to determine the gamma D_{10}-values. The Gamma D_{10}-values were calculated from the straight portion of the survival curve depicting the $\log_{10}$ of viable spores against the dose in kGy.

CHAPTER 4

RESULTS

4.1 Preliminary experiments

The effects of 250 mg/l Cl_2 wash and blanching in four changes of water at 77^{o}C for 6 min on the microbial load of spinach, are shown in Table 2 and Figure 1. Analysis showed that there were no detectable levels (< 10 cfu/g) of aerobic mesophilic spore-formers in the raw spinach.

Table 2 Effects of pre-processing on the microbial load of spinach

Treatments	Total plate count (log_{10} cfu/g)
Raw	6.79 b[1] (0.12)[2]
↓	
Rinse in 250 mg/l chlorine solution	3.68 a (0.13)
↓	
Blanch in first change of water (77^{o}C for 6 min)	3.74 a (0.14)
↓	
Blanch in second change of water (77^{o}C for 6 min)	3.57 a (0.17)
↓	
Blanch in third change of water (77^{o}C for 6 min)	3.39 a (0.12)
↓	
Blanch in fourth change of water (77^{o}C for 6 min)	3.29 a (0.17)

[1] Mean values with a different letter are significantly different ($p \leq 0.05$)
[2] Numbers in brackets represent the standard deviation

Chlorine at a concentration of 250 mg/l reduced the microbial load of the spinach significantly (3 log_{10} cycle). Blanching did not reduce the total plate counts significantly following the Cl_2 wash. Blanching in two changes of water at 77^{o}C for 6 min was chosen to be used in subsequent experiments, as blanching in further changes of water led to significant reductions in total solids (Figure 1).

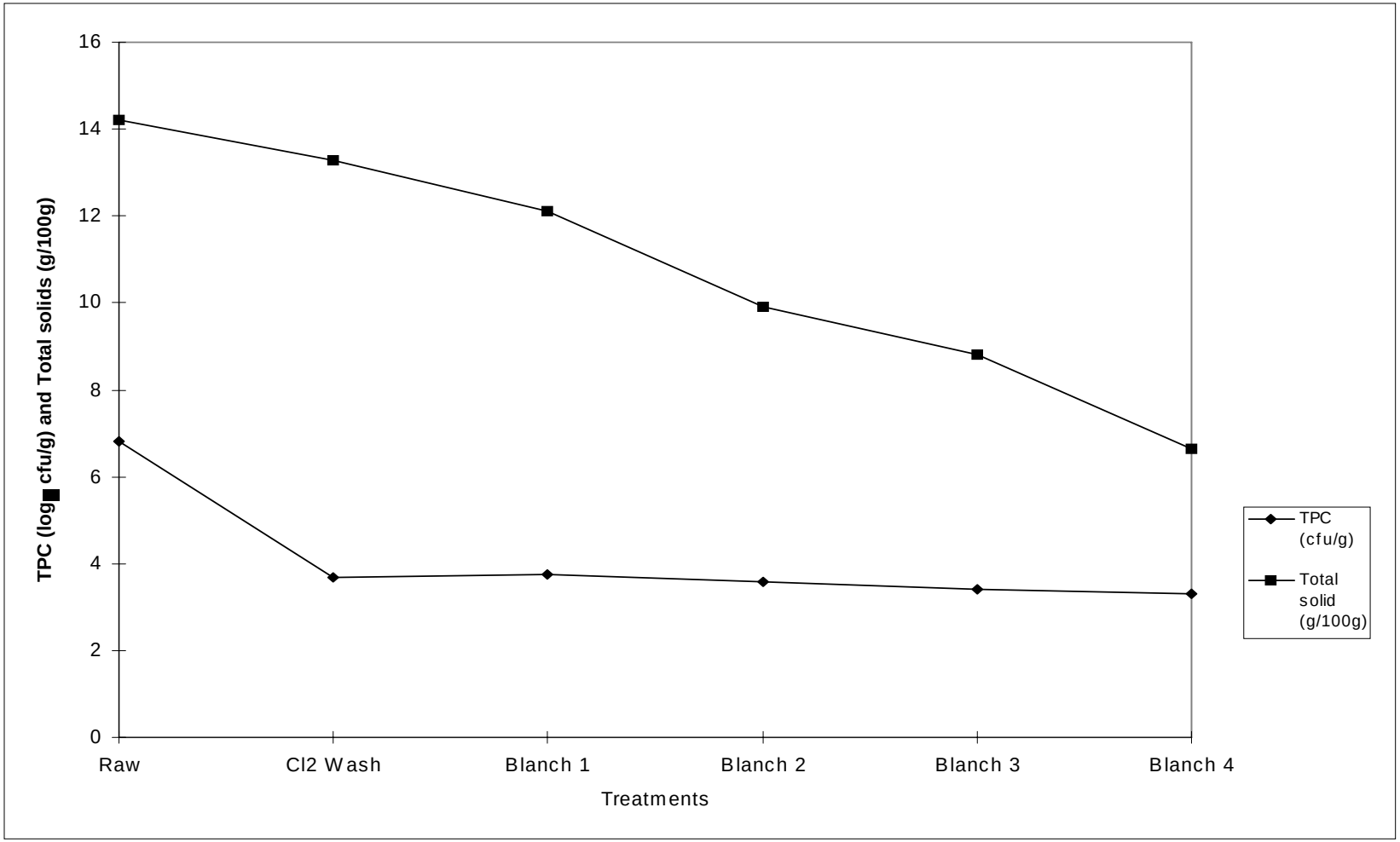

Figure 2 Effects of Cl_2 wash and blanching in four changes of water at 77^{o}C for 6 min on the TPC (log_{10} cfu/g) and total solids content of spinach

4.2 Phase 1: Effects of MAP in combination with irradiation at different doses on microbial load in a RTE sorghum porridge and spinach meal

Table 3 shows the effects of MAP in combination with irradiation on the microbial load, as measured by TPC (log_{10} cfu/g), in the RTE meal. Analysis showed that there were no detectable levels (< 10 cfu/g) of aerobic mesophilic spore-formers in the RTE meal.

Table 3 Effect of a combination of irradiation and MAP on the TPC (log_{10} cfu/g) in a RTE meal immediately after irradiation (0 to2 hr) at ambient temperature (20 to 25°C)

Target irradiation dose (kGy)	Control	MAP 1[1]	MAP 2[2]	Irradiation effect[3]
0	2.41 (1.18)[4]	1.81 (1.26)	1.64 (0.98)	1.95 b (0.41)
2	< 1.00 (0.00)	< 1.00 (0.00)	< 1.00 (0.00)	< 1.00 a (0.00)
4	< 1.00 (0.00)	< 1.00 (0.00)	< 1.00 (0.00)	< 1.00 a (0.00)
6	< 1.00 (0.00)	< 1.00 (0.00)	< 1.00 (0.00)	< 1.00 a (0.00)
8	< 1.00 (0.00)	< 1.00 (0.00)	< 1.00 (0.00)	< 1.00 a (0.00)
10	< 1.00 (0.00)	< 1.00 (0.00)	< 1.00 (0.00)	< 1.00 a (0.00)
MAP effect[5]	1.36 a (0.58)	1.27 a (0.33)	1.21 a (0.25)	

1 MAP 1: 84.5% N_2 + 15.5% CO_2;
2 MAP 2: 82.3% N_2, 15.9% CO_2 + 1.8% O2
3 Mean values in column with a different letter are significantly different ($p \leq 0.05$)
4 Standard deviations are given in brackets
5 Mean values in row with a different letter are significantly different ($p \leq 0.05$)

Table 3 Cont'd Analysis of variance for TPC (log_{10} cfu/g) in the RTE meal

Source of variation	DF	Mean square	F	P
MAP	2	0.099	0.180	0.836
Irradiation	5	0.843	1.535	0.204
MAP * Irradiation	10	0.122	0.223	0.992
Residual	36	0.228		

DF = degrees of freedom, F = F-test, P = significance level

Statistical analysis of the data obtained from the experiment indicated that overall, MAP did not have any effect on the microbial load in the RTE meal. Irradiation at 2 kGy (target dose) significantly ($p \leq 0.05$) reduced the microbial load in the meal, but there was no significant difference between the effects of irradiation at 2 kGy (target dose) and any of the other irradiation target doses (4, 6, 8 and 10 kGy). There was no significant interaction between MAP and irradiation on the TPC in the RTE meal as illustrated in the analysis of variance section of Table 3.

Table 4 shows the effect of irradiation at different doses under different modified atmosphere packaging conditions on the inactivation of *C. sporogenes* (log_{10} cfu/g) inoculated into the RTE meal.

Table 4. Effect of a combination of irradiation and MAP on the inactivation of *C. sporogenes* (log_{10} cfu/g) inoculated into a RTE meal

Target irradiation dose (kGy)	Control	MAP 1[1]	MAP 2[2]	Irradiation effect[3]
0	7.25 (0.35)[4]	5.88 (0.62)	5.76 (0.54)	6.30 e (0.83)
2	5.42 (0.15)	5.48 (0.27)	5.70 (0.62)	5.54 d (0.15)
4	4.49 (0.07)	4.98 (0.64)	5.32 (0.43)	4.93 c (0.42)
6	3.71 (0.34)	3.62 (0.30)	4.08 (0.57)	3.80 b (0.34)
8	3.92 (0.26)	3.94 (0.34)	3.86 (0.25)	3.91 b (0.04)
10	2.82 (0.31)	3.08 (0.31)	3.28 (0.35)	3.06 a (0.23)
MAP effect[5]	4.60 a (1.56)	4.50 a (1.11)	4.67 a (1.06)	

1 MAP 1: 84.5% N_2 + 15.5% CO_2;
2 MAP 2: 82.3% N_2, 15.9% CO_2 + 1.8% O2
3 Mean values in column with a different letter are significantly different ($p \leq 0.05$)
4 Standard deviations are given in brackets
5 Mean values in row with a different letter are significantly different ($p \leq 0.05$)

Analysis of variance for *C. sporogenes* (log_{10} cfu/g) inoculated into the RTE meal

Source of variation	DF	Mean square	F	P
MAP	2	0.207	0.909	0.412
Irradiation	5	14.604	64.148	0.000
MAP * Irradiation	10	0.608	2.671	0.015
Residual	36	0.228		

DF = degrees of freedom, F = F-test, P = significance level

Overall, MAP had no effect on the inactivation and/or proliferation of *C. sporogenes* inoculated into the RTE meal. However, a comparison of gamma D_{10}-values obtained for the different MAP conditions in Figure 3 shows lower gamma D_{10}-value for MAP 0 (control) (2.41 kGy) than for MAP 1 (84.5% N_2 + 15.5% CO_2) (3.23 kGy) and MAP 2 (82.3% N_2, 15.9% CO_2 + 1.8% O_2) (3.63 kGy). The gamma D_{10}-values for *C. sporogenes* obtained under conditions of MAP 0 (control) were as expected but those for MAP 1 (84.5% N_2 + 15.5% CO_2) and MAP 2 (82.3% N_2, 15.9% CO_2 + 1.8% O_2), were higher than expected.

Irradiation reduced *C. sporogenes* inoculated into the RTE meal significantly ($p \leq 0.001$) (more than 3.0 $\log_{10}$ cycles between target doses 0 to 10 kGy). There was a significant ($p \leq 0.05$) interaction between the effects of MAP and irradiation on the inactivation and/or proliferation of *C. sporogenes* inoculated into the RTE meal, as can be seen from the analysis of variance section of Table 4. However, combining MAP and irradiation appeared not to have a synergistic effect. In fact, from Figure 3 it can be seen that the combination of MAP and irradiation had higher gamma D_{10}-values than the irradiation air control.

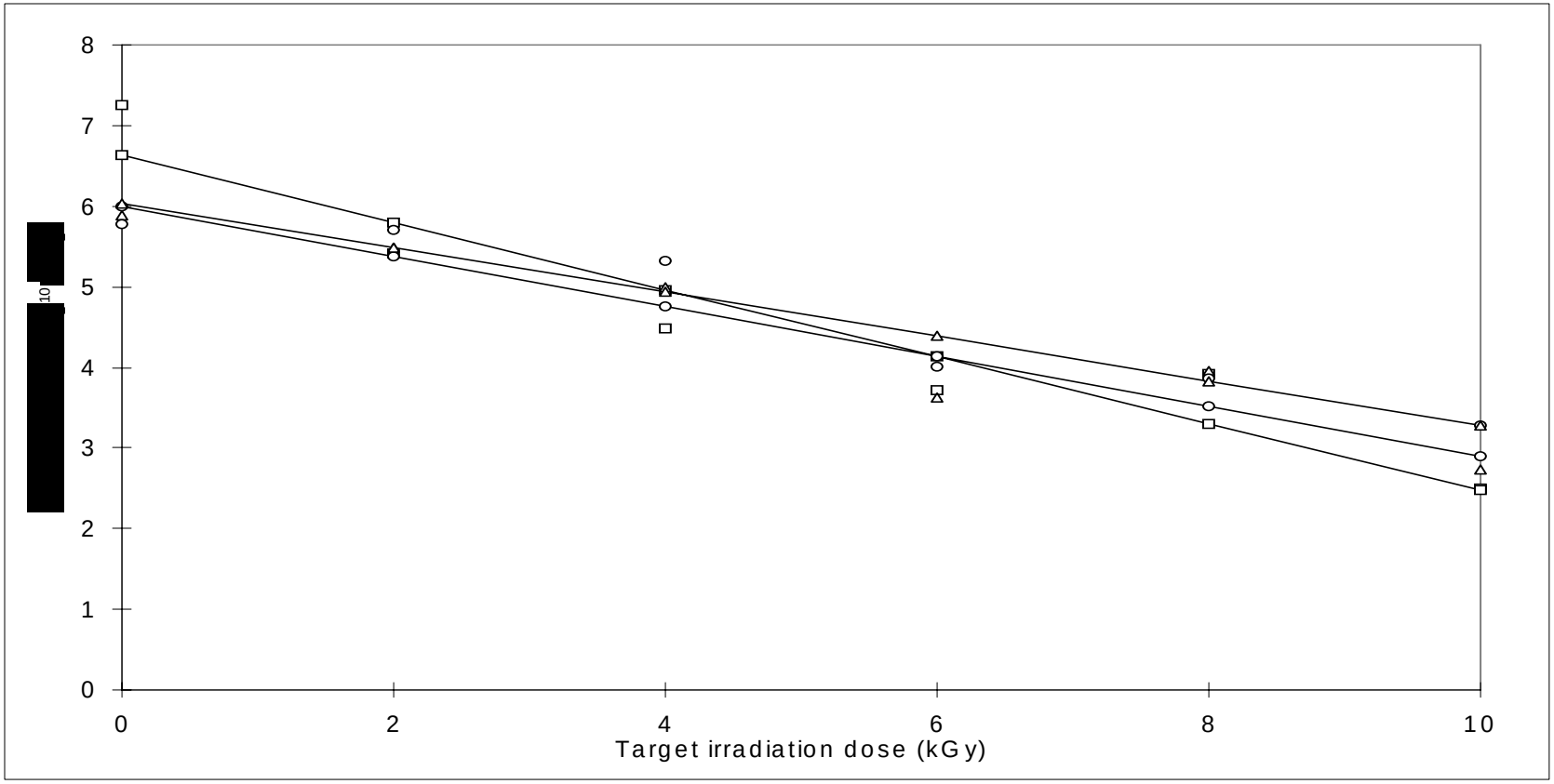

Figure 3 Effects of a combination of different irradiation dose levels and MAP conditions on the inactivation of *C. sporogenes* (log_{10} cfu/g) inoculated into the RTE sorghum porridge and spinach (morôgo) meal.

□ MAP 0: Control (air) (R^2 = 0.95; gamma D_{10}-value = 2.41 kGy)
Δ MAP 1: 84.5% N_2 + 15.5% CO_2 (R^2 = 0.96; gamma D_{10}-value = 3.23 kGy)
o MAP 2: 82.3% N_2, 15.9% CO_2 + 1.8% O_2 (R^2 = 0.96; gamma D_{10}-value = 3.63 kGy)

It was also expected that increasing the irradiation dose level from 6 to 8 kGy (target doses) would decrease the counts of *C. sporogenes* in the RTE meal. However, no such effect was found. Reasons for this unexpected result were not apparent at first. It was suggested that interruptions during irradiation and time spent at ambient temperature by the RTE meal samples during and after irradiation processing may have contributed towards these unexpected results. Samples (for treatments using 8 and 10 kGy irradiation target doses) were irradiated to 6 kGy (target dose) on day 1, left overnight at ambient temperatures before being irradiated to 8 and 10 kGy (target dose) the following morning. All samples were kept at ambient temperatures until analyzed. Subsequently, it was decided to repeat Phase 1 (i.e. validation of Phase 1) using stricter control measures on the irradiation processing of the samples (i.e. continuous uninterrupted irradiation processing and prompt analysis for microbial activity after irradiation processing).

4.2.1 Validation of Phase 1

Table 5 and Figure 4 show the results of the validation of Phase 1 with stricter control measures in place during irradiation processing in order to prevent any interruptions (up to 14 h) during the irradiation (to a target dose of 20 kGy with 2 kGy intervals) of the RTE meal. MAP had no significant effect, whilst irradiation processing reduced the *C. sporogenes* counts inoculated into the RTE meal significantly ($p \leq 0.001$). The analysis of variance (Table 5) showed that there was no interaction between MAP and irradiation regarding the inactivation and proliferation of *C. sporogenes* inoculated into the RTE meal.

Table 5 Validation of the effect of a combination of irradiation and MAP on the inactivation of *C. sporogenes* (log_{10} cfu/g) inoculated into the RTE meal

Target Irradiation dose (kGy)	Actual irradiation dose (kGy)	Control	MAP 1[1]	MAP 2[2]	Irradiation effect[3]
0	0	7.38 (2.03)[4]	7.42 (2.73)	7.31 (0.34)	7.37 f (0.06)
2	1.09	6.52 (0.07)	6.70 (0.10)	6.48 (0.17)	6.57 ef (0.12)
4	2.68	6.67 (0.25)	6.39 (0.18)	6.20 (0.16)	6.42 e (0.24)
6	5.78	5.29 (2.13)	5.25 (1.83)	5.31 (0.16)	5.28 d (0.03)
8	8.91	4.41 (1.09)	4.56 (0.23)	4.52 (0.14)	4.50 d (0.08)
10	11.52	2.93 (0.64)	2.99 (0.14)	2.99 (0.23)	2.97 c (0.03)
12	13.43	2.22 (0.12)	2.16 (0.23)	2.27 (0.52)	2.22 b (0.06)
14	14.99	1.88 (0.34)	1.68 (0.29)	1.90 (0.24)	1.82 b (0.12)
16	16.21	< 1.00 (0.23)	1.34 (0.34)	1.08 (0.29)	1.14 a (0.18)
18	17.41	0.90 (0.23)	0.70 (0.17)	< 1.00 (0.00)	0.53 a (0.47)
20	18.38	< 1.00 (0.00)	< 1.00 (0.00)	< 1.00 (0.00)	< 1.00 a (0.00)
MAP effect[5]		3.56 a (2.60)	3.56 a (2.61)	3.46 a (2.64)	

[1] MAP 0 (control); MAP 1 (84.5% N_2 + 15.5% CO_2); MAP 2 (82.3% N_2, 15.9% CO_2 + 1.8% O_2)
[2] Mean values in column with a different letter are significantly different ($p \leq 0.05$)
[3] Standard deviations are given in brackets
[4] Mean values in row with a different letter are significantly different ($p \leq 0.05$)

Table 5 cont'd

Analysis of variance for *C. sporogenes* (log_{10} cfu/g) inoculated into the RTE meal (validation)

Source of variation	DF	Mean square	F	P
MAP	2	0.424	0.423	0.655
Irradiation	10	196.410	196.202	0.000
MAP * Irradiation	20	1.360	1.359	0.142
Residual	297	1.001		

DF = degrees of freedom, F = F-test, P = significance level

Actual irradiation doses achieved were different from the target dose levels. Although an increase in irradiation followed the expected trend in inactivating *C. sporogenes* in the RTE meal, the inactivation effect of the following target dose levels did not differ significantly: 0 and 2 kGy; 2 and 4 kGy, 6 and 8 kGy, 12 and 14 kGy; 16, 18 and 20 kGy.

There was no MAP effect on the *C. sporogenes* inoculated into the RTE meal, while irradiation reduced *C. sporogenes* in the RTE meal significantly ($p \leq 0.001$). There were no interactions between MAP and irradiation. The gamma D_{10}-values required to inactivate 90 % of the population for MAP 0, MAP 1 and MAP 2 were 2.60 kGy, 2.59 kGy and 2.58 kGy respectively.

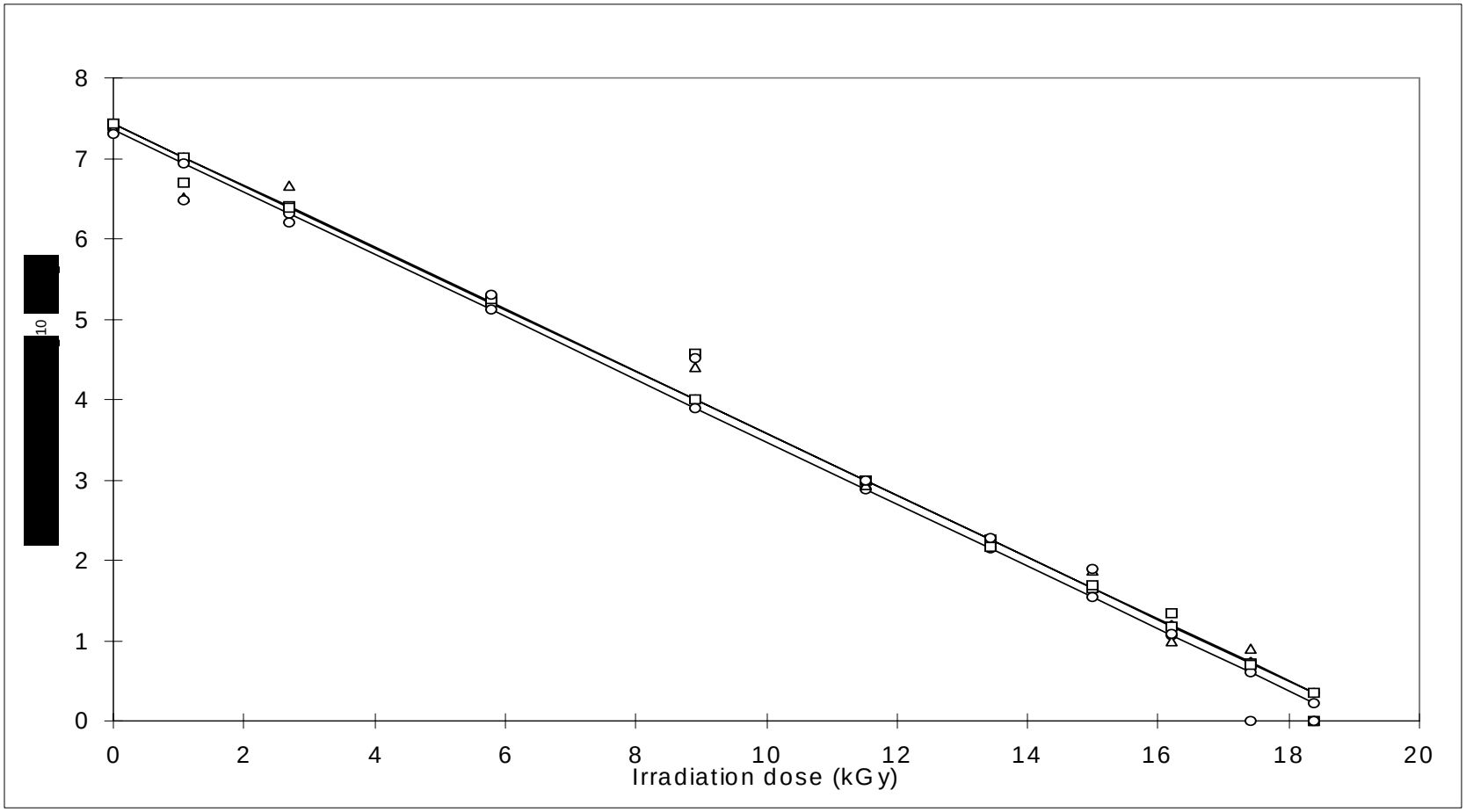

Figure 4 Validation of the effect of different irradiation dose levels in combination with different MAP conditions on the inactivation of *C. sporogenes* (log_{10} cfu/g) inoculated into the RTE sorghum porridge and spinach (morôgo) meal.

□ MAP 0: Control (air) (R^2 = 0.98; gamma D_{10}-value = 2.60 kGy)
Δ MAP 1: 84.5% N_2 + 15.5% CO_2 (R^2 = 0.99; gamma D_{10}-value = 2.59 kGy)
o MAP 2: 82.3% N_2, 15.9% CO_2 + 1.8% O_2 (R^2 = 0.98; gamma D_{10}-value = 2.58 kGy)

4.3 Phase 2: Effect of irradiation in combination with MAP 1 on the shelf-life (TPC) and safety (*C. sporogenes counts*) of the RTE meal stored at both 5°C and 37°

In this phase, the effect of irradiation at 10 kGy (target dose) in combination with MAP 1 (84.5% N_2 + 15.5% CO_2) on the shelf-life (as measured by TPC) safety (as measured by *C. sporogenes* count) of the RTE meal stored at both 5°C and 37°C for seven days was investigated.

Table 6 and Figure 5 show the effects of the combination of MAP and irradiation on the survival and growth of micro-organisms in the RTE meal stored at 5°C for a period of 7 d.

Table 6 Effects of the combination of MAP 1 and irradiation at a target dose level of 10 kGy on the survival and growth of TPC (log_{10} cfu/g) in a RTE meal stored at 5°C for 7d

Time of storage at 5°C (days)	Control	MAP 1[1]	Irradiation	Irradiation + MAP 1	Time effect[2]
1	4.06 (1.32)[3]	2.40 (0.65)	< 1.00 (0.00)	< 1.00 (0.00)	2.11 a (1.45)
3	4.59 (0.18)	3.38 (0.19)	< 1.00 (0.00)	< 1.00 (0.00)	2.48 a (1.79)
5	5.96 (0.51)	4.80 (0.77)	< 1.00 (0.00)	< 1.00 (0.00)	3.19 b (2.57)
7	6.76 (0.55)	5.47 (1.65)	< 1.00 (0.00)	< 1.00 (0.00)	3.55 b (3.00)
MAP effect[4]	3.16 b (MAP 0)		2.50 a (MAP 1)		
Irradiation effect[4]	4.66 b		< 1.00 a		

1 MAP 1: 84.5% N_2 + 15.5% CO_2
2 Mean values in column with a different letter are significantly different ($p \leq 0.05$)
3 Standard deviations are given in brackets
4 Mean values in rows with a different letter are significantly different ($p \leq 0.05$)

Table 6 cont'd

Analysis of variance for TPC (log_{10} cfu/g) in the RTE meal stored at 5^{o}C for 7 d

Source of variation	DF	Mean square	F	P
Day	3	15.486	40.409	0.000
MAP	1	15.939	41.592	0.000
Irradiation	1	482.941	1260.184	0.000
Day * MAP	3	0.118	0.307	0.820
Day * Irradiation	3	15.495	40.409	0.000
MAP * Irradiation	1	15.939	41.592	0.000
Day * MAP * Irradiation	3	0.118	0.307	0.820
Residual	128	0.383		

DF = degrees of freedom, F = F-test, P = significance level

Overall, MAP and irradiation processing reduced the survival and growth of micro-organisms significantly ($p \leq 0.001$). Storage time (days) also had a significant ($p \leq 0.001$) effect on the proliferation of TPC as shown in the analysis of variance section of Table 6. The longer the storage times, the higher the TPC in the RTE meal. Statistical analysis of the data shows there was a significant ($p \leq 0.001$) interaction between MAP and irradiation. However, it should be noted that there was no measurable microbial growth in the irradiated, MAP 1 treatment or the irradiated, air control whereas there was noticeable microbial activity in the MAP treated RTE meal samples. As a result it can be assumed that the reduction of TPC is solely due to the effects of irradiation.

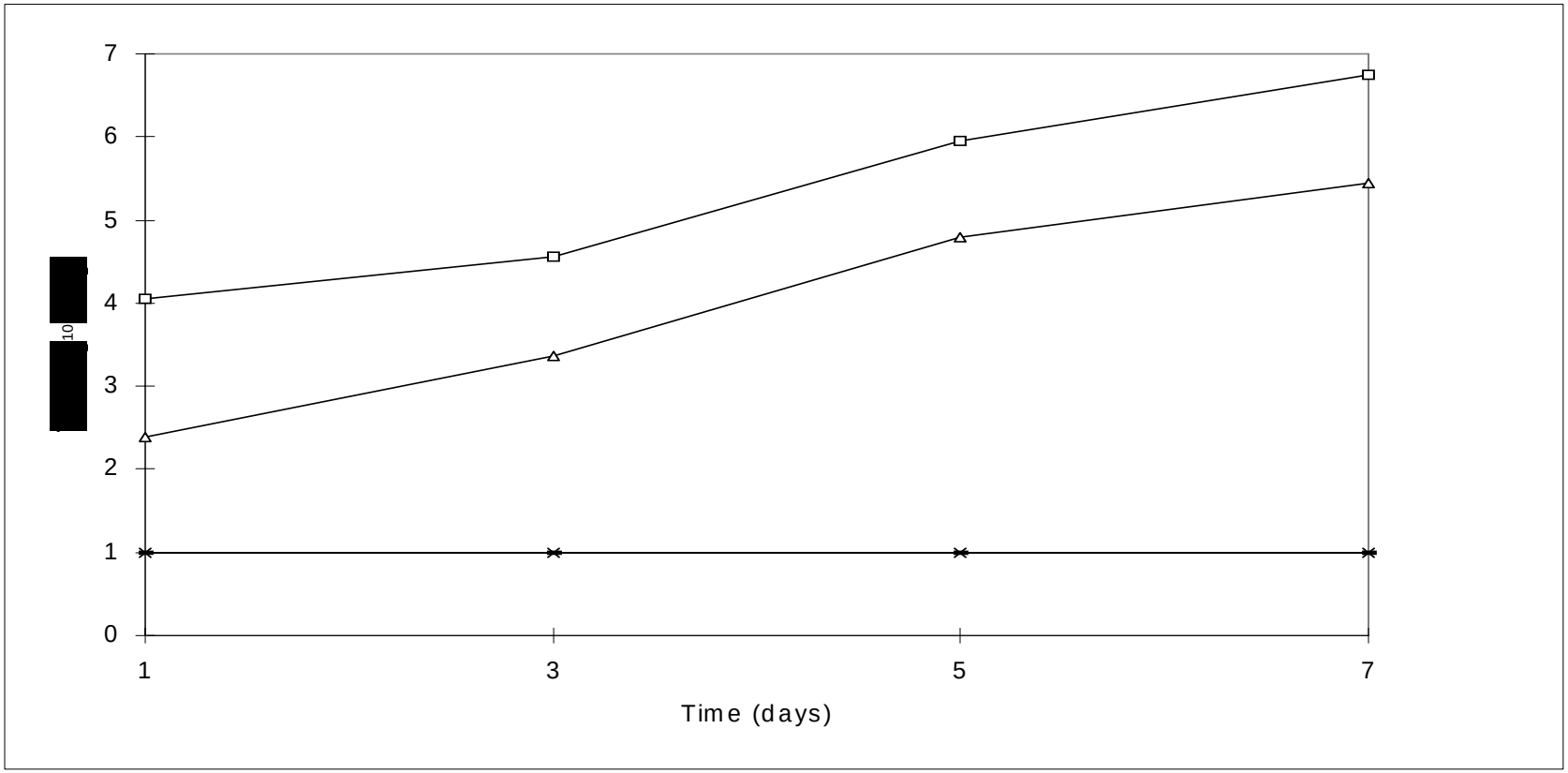

Figure 5 Effects of a combination of irradiation and MAP on TPC (log_{10} cfu/g) in the RTE meal over a storage period of 7 d at 5°C

□: Irradiation at 0 kGy, MAP 0 (air), and storage at 5°C (Control)

Δ: Irradiation at 0 kGy, MAP 1 (84.5% N_2 & 15.5% CO_2), and storage at 5°C (MAP)

–: Irradiation at 10 kGy, MAP 0 (air), and storage at 5°C (Irradiation)

×: Combination of irradiation at 10 kGy, MAP 1 (84.5% N_2 & 15.5% CO_2), and storage at 5°C (MAP + Irradiation)

Figure 6 shows MAP 0 (control) and MAP 1 (84.5% N_2 + 15.5% CO_2) packaged RTE meal samples after 2 d at 37^{o}C

Figure 6. MAP 0 (control) (left) and MAP 1 (84.5% N_2 + 15.5% CO_2) (right) packaged RTE meal samples after 2 d at 37^{o}C

Table 7 and Figure 7 show the effects of MAP and irradiation on TPC in the RTE meal during a storage period of 7 d at 37^{o}C as compared to a control.

Table 7 Effects of the combination of MAP and irradiation to a target dose of 10 kGy on the survival and growth of TPC (log_{10} cfu/g) in a RTE meal stored at 37°C for 7 d

Time of storage At 37°C (days)	Control	MAP 1[1]	Irradiation	Irradiation + MAP 1	Time effect[2]
1	9.10 (0.06)[3]	7.69 (0.47)	< 1.00 (0.00)	< 1.00 (0.00)	4.70 a (4.31)
3	10.00 (0.00)	10.00 (0.00)	4.19 (0.00)	3.02 (0.53)	6.79 b (3.72)
5	10.00 (0.00)	10.00 (0.00)	8.63 (0.50)	3.48 (0.25)	8.02 c (3.10)
7	10.00 (0.00)	10.00 (0.00)	10.00 (0.00)	2.64 (0.20)	8.16 c (3.48)
MAP effect[4]	7.86 b (MAP 0)		5.97 a (MAP 1)		
Irradiation effect[4]	9.60 b		4.24 a		

[1] MAP 1: 84.5% N_2 + 15.5% CO_2
[2] Mean values in column with a different letter are significantly different ($p \leq 0.05$)
[3] Standard deviations are given in brackets
[4] Mean values in rows with a different letter are significantly different ($p \leq 0.05$)

Analysis of variance for TPC (log_{10} cfu/g) in the RTE meal stored at 37°C for 7 d

Source of variation	DF	Mean square	F	P
Day	3	92.367	1613.25	0.000
MAP	1	129.106	2254.93	0.000
Irradiation	1	1035.499	18085.67	0.000
Day * MAP	3	20.462	357.38	0.000
Day * Irradiation	3	29.993	523.85	0.000
MAP * Irradiation	1	85.362	1490.91	0.000
Day * MAP * Irradiation	3	35.043	612.05	0.000
Residual	128	0.057		

DF = degrees of freedom, F = F-test, P = significance level

Both MAP and irradiation processing reduced the overall survival and growth of micro-organisms significantly ($p \leq 0.001$). TPC for the non-irradiated samples increased to 10^{10} cfu/g by day three of storage, whereas microbial counts for the MAP-irradiation combination treated samples increased slightly, but remained less than 10^{4} cfu/g. Microbial counts in samples treated with irradiation at 10 kGy (target dose) reached 10^{10} cfu/g only after the 7 th day of storage. MAP initially reduced TPC for the non-irradiated samples. However, by day three MAP had no effect. Storage time (days) at 37 °C also had a significant ($p \leq 0.001$) effect on the activity of micro-organisms. The TPC increased significantly with longer storage time.

There were significant interactions between storage time (days) and MAP ($p \leq 0.001$); storage time (days) and irradiation ($p \leq 0.001$); MAP and irradiation ($p \leq 0.001$); and storage time (days), MAP and irradiation ($p \leq 0.001$). In this case, the interaction between MAP and irradiation was synergistic i.e. TPC and their proliferation in the RTE meal were significantly reduced compared to irradiation and MAP as separate treatments, during the storage period.

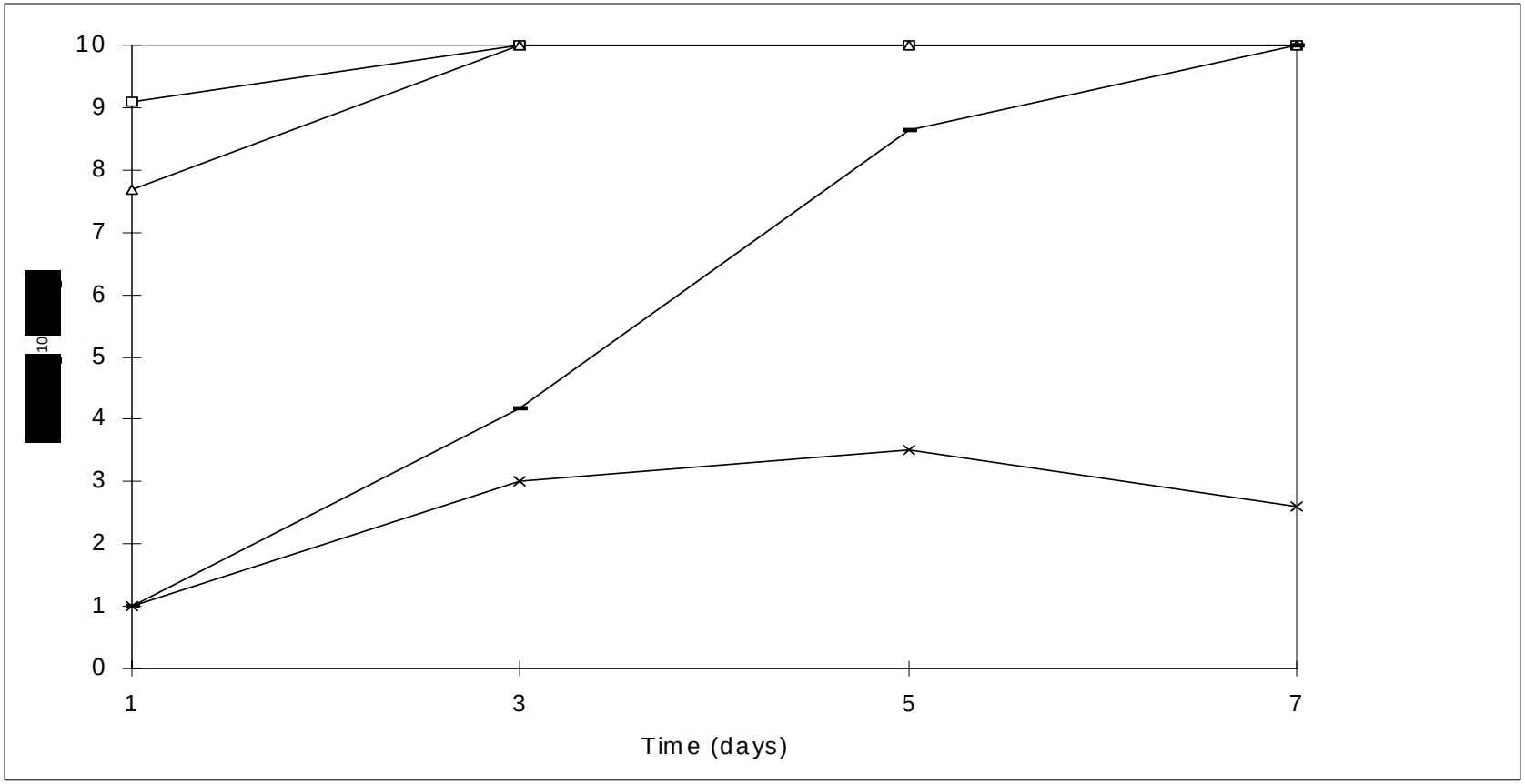

Figure 7 Effects of a combination of irradiation and MAP on TPC (log_{10} cfu/g) the RTE sorghum porridge and spinach (morôgo) meal over a storage period of 7 d at 37°C

□: Irradiation at 0 kGy, MAP 0 (air), and storage at 37°C (Control)

Δ: Irradiation at 0 kGy, MAP 1 (84.5% N_2 & 15.5% CO_2), and storage at 37°C (MAP)

–: Irradiation at 10 kGy, MAP 0 (air), and storage at 37°C (Irradiation)

×: Combination of irradiation at 10 kGy, MAP 1 (84.5% N_2 & 15.5% CO_2), and storage at 37°C (MAP + Irradiation)

Tables 8 and 9 and Figures 8 and 9 show the effect of MAP and irradiation on the inactivation and/or proliferation of *C. sporogenes* inoculated into the RTE meal and stored at 5°C and 37°C, respectively, for a period of 7 d.

Table 8 Effects of the combination of MAP and irradiation to a target dose of 10 kGy on the inactivation and proliferation of *C. sporogenes* (log_{10} cfu/g) inoculated into a RTE sorghum porridge and spinach (morôgo) meal stored at 5°C for 7 d

Time of storage At 5°C (days)	Control	MAP 1[1]	Irradiation	Irradiation + MAP 1	Time effect[2]
1	7.77 (0.49)[3]	7.34 (0.12)	2.64 (0.06)	3.64 (0.19)	5.34 a (2.59)
3	7.56 (0.11)	7.42 (0.10)	3.05 (0.52)	3.30 (0.97)	5.32 a (2.49)
5	7.01 (0.78)	6.16 (1.14)	4.74 (0.53)	4.36 (0.71)	5.57 ab (1.23)
7	7.28 (0.62)	5.88 (1.70)	5.09 (1.93)	5.45 (1.77)	6.08 b (1.11)
MAP effect[4]	5.62 a (MAP 0)		5.43 a (MAP 1)		
Irradiation effect[4]	7.03 b		4.02 a		

1 MAP 1: 84.5% N_2 + 15.5% CO_2
2 Mean values in column with a different letter are significantly different ($p \leq 0.05$)
3 Standard deviations are given in brackets
4 Mean values in rows with a different letter are significantly different ($p \leq 0.05$)

Table 8 cont'd

Analysis of variance for *C. sporogenes* (log_{10} cfu/g) in the RTE meal stored at 5^{o}C for 7 d

Source of variation	DF	Mean square	F	P
Day	3	2.717	3.028	0.032
MAP	1	1.234	1.375	0.243
Irradiation	1	326.194	363.550	0.000
Day * MAP	3	1.560	1.739	0.162
Day * Irradiation	3	22.819	25.432	0.000
MAP * Irradiation	1	8.826	9.837	0.002
Day * MAP * Irradiation	3	1.142	1.272	0.287
Residual	128	0.897		

DF = degrees of freedom, F = F-test, P = significance level

Irradiation at 10 kGy (target dose), reduced initial counts of *C. sporogenes* inoculated into the RTE meal significantly ($p \leq 0.001$) as compared to no irradiation (0 kGy) but did not prevent the surviving spores from germinating and growing. At 5^{o}C, MAP did not have any statistically significant effect on the inactivation and/or proliferation of *C. sporogenes* inoculated into the RTE meal. Storage time (days) had a significant ($p \leq 0.05$) effect on the proliferation of *C. sporogenes*, with counts increasing by approximately 2 log_{10} cycles for the irradiated samples and remaining more or less constant for the unirradiated samples during the 7 d storage period, Significant interactions were observed between storage time (days) and irradiation ($p \leq 0.001$) as well as irradiation and MAP ($p \leq 0.05$). However combining MAP and irradiation did not in fact reduce *C. sporogenes* counts and their proliferation in the RTE meal during the storage period at 5^{o}C.

Table 9 Effects of the combination of MAP and irradiation to a target dose of 10 kGy on the inactivation and proliferation of *C. sporogenes* (log_{10} cfu/g) inoculated into a RTE meal stored at 37°C for 7 d

Time of storage at 37°C (days)	Control	MAP 1[1]	Irradiation	Irradiation + MAP 1	Time effect[2]
1	8.66 (0.14)[3]	8.25 (0.11)	2.47 (0.10)	3.43 (0.20)	5.69 a (3.20)
3	10.00 (0.00)	10.00 (0.00)	3.30 (1.22)	3.14 (0.57)	6.61 b (3.91)
5	10.00 (0.00)	10.00 (0.00)	6.19 (2.87)	4.70 (0.66)	7.72 c (2.70)
7	10.00 (0.00)	10.00 (0.00)	5.65 (3.27)	4.61 (2.96)	7.56 c (2.84)
MAP effect[4]	7.03 a		6.75 a		
Irradiation effect[4]	9.61 b		4.17 a		

[1] MAP 1: 84.5% N_2 + 15.5% CO_2
[2] Mean values in column with a different letter are significantly different ($p \leq 0.05$)
[3] Standard deviations are given in brackets
[4] Mean values in rows with a different letter are significantly different ($p \leq 0.05$)

Analysis of variance for *C. sporogenes* (log_{10} cfu/g) in the RTE meal stored at 37°C for 7 d

Source of variation	DF	Mean square	F	P
Day	3	31.964	17.032	0.000
MAP	1	2.803	1.494	0.224
Irradiation	1	1066.295	568.171	0.000
Day * MAP	3	1.799	0.959	0.414
Day * Irradiation	3	8.978	4.784	0.003
MAP * Irradiation	1	1.104	0.588	0.444
Day * MAP * Irradiation	3	3.552	1.893	0.134
Residual	128	1.877		

DF = degrees of freedom, F = F-test, P = significance level

At 37^{o}C (Table 9 and Figure 9), MAP had no effect on the inactivation and/or proliferation of *C. sporogenes* in the RTE meal during the 7 d storage period. Storage time (days) had a significant effect on the proliferation of *C. sporogenes* in the RTE meal, with counts increasing for both the irradiated and unirradiated samples during the 7 d storage period. There was no interaction between MAP and irradiation.

4.3.1 Validation of phase 2

Phase 2 was repeated over a longer storage time period using stricter control measures in order to prevent any interruptions (up to 14 h) during the irradiation processing of samples. Tables 10 and 11 and Figure 10 show the combined effects of MAP 1 (84.5% N_2 & 15.5% CO_2) and irradiation at 11.52 kGy (target dose of 10 kGy) on the inactivation and/or proliferation of *C. sporogenes* inoculated into the RTE meal and stored at 5^{o}C and 37^{o}C for a period of 13 d.

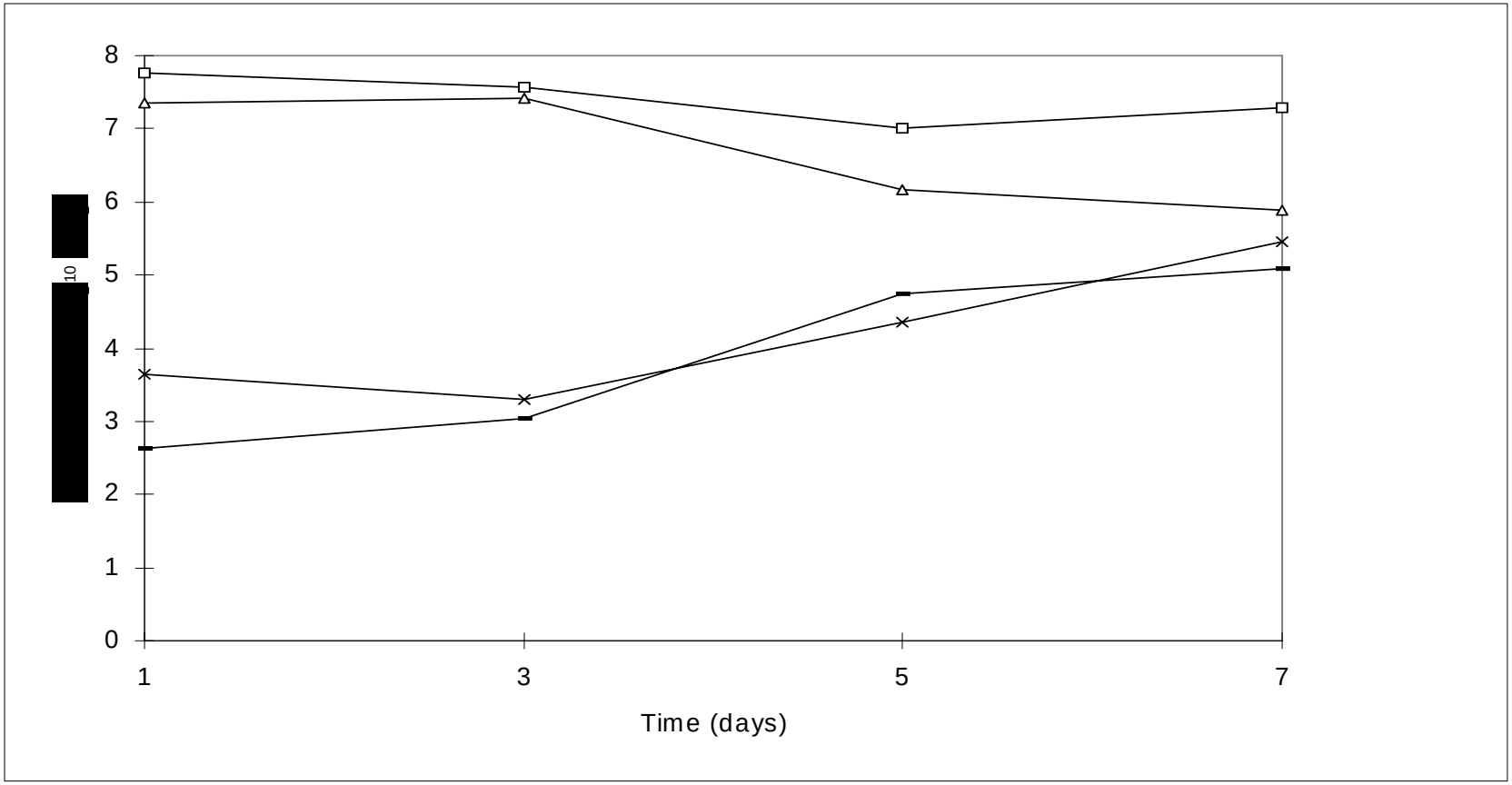

Figure 8 Effects of the combination of MAP and irradiation at 10 kGy on the inactivation and proliferation of *C. sporogenes* (log_{10} cfu/g) inoculated into a RTE sorghum porridge and spinach (morôgo) meal stored for 7 d at 5°C

□: Irradiation at 0 kGy, MAP 0 (air), and storage at 5°C (Control)

Δ: Irradiation at 0 kGy, MAP 1 (84.5% N_2 & 15.5% CO_2), and storage at 5°C (MAP)

–: Irradiation at 10 kGy, MAP 0 (air), and storage at 5°C (Irradiation)

×: Combination of irradiation at 10 kGy, MAP 1 (84.5% N_2 & 15.5% CO_2), and storage at 5°C (MAP + Irradiation)

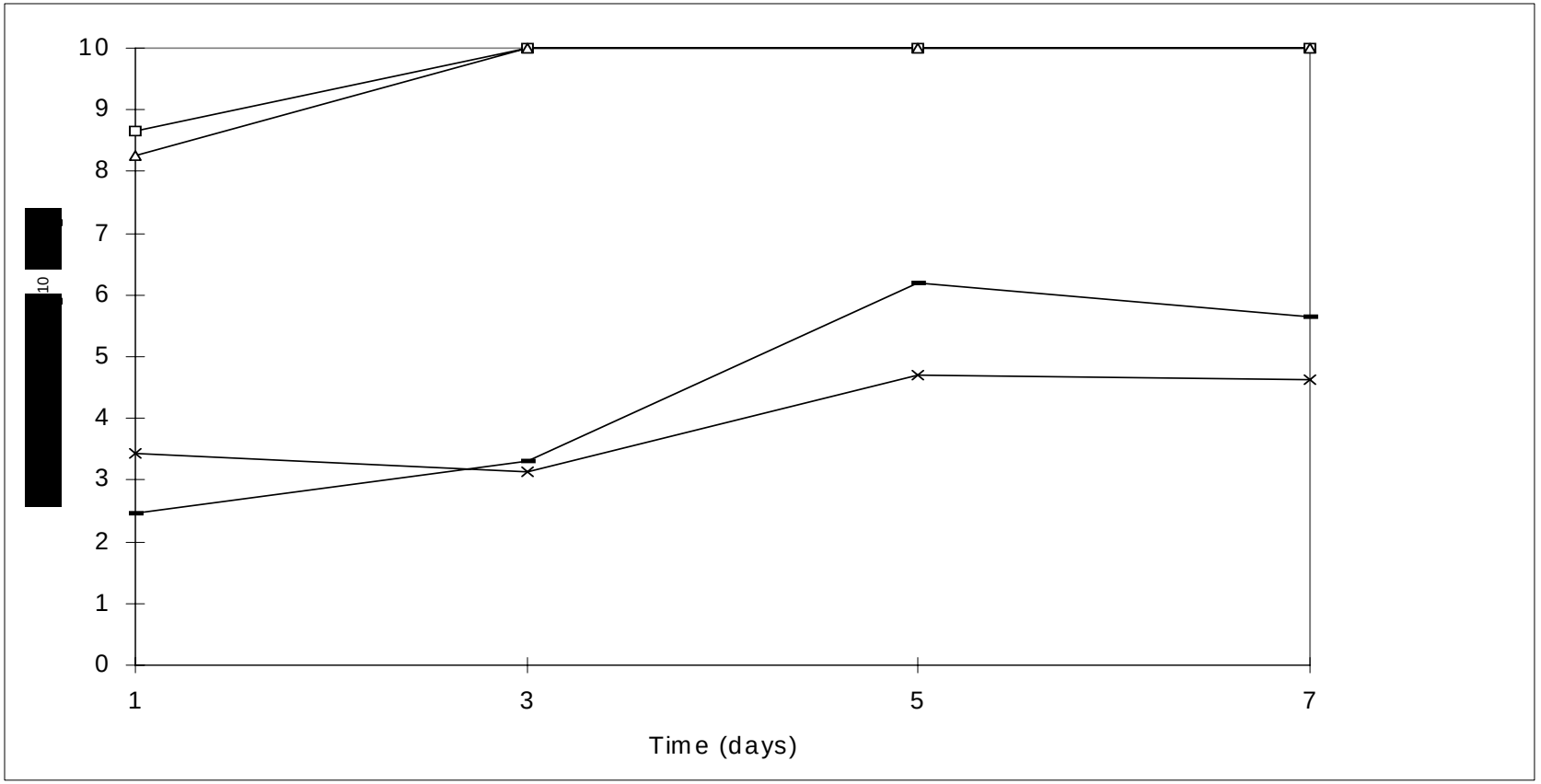

Figure 9 Effects of the combination of irradiation and MAP on the inactivation and proliferation of *C. sporogenes* (log_{10} cfu/g) inoculated into the RTE sorghum porridge and spinach (morôgo) meal over a storage period of 7 d at 37°C

□: Irradiation at 0 kGy, MAP 0 (air), and storage at 37°C (Control)

Δ: Irradiation at 0 kGy, MAP 1 (84.5% N_2 & 15.5% CO_2), and storage at 37°C (MAP)

–: Irradiation at 10 kGy, MAP 0 (air), and storage at 37°C (Irradiation)

×: Combination of irradiation at 10 kGy, MAP 1 (84.5% N_2 & 15.5% CO_2), and storage at 37°C (MAP + Irradiation)

Table 10 Validation of the effects of the combination of MAP and irradiation at 11.52 kGy on the inactivation and proliferation of *C. sporogenes* (log_{10} cfu/g) inoculated into a RTE meal stored at 5°C for 13 d

Time of storage at 5°C (days)	Irradiation	Irradiation + MAP 1[1]	Time effect[2]
0	3.22 (0.09)[3]	3.30 (0.09)	3.26 a (0.06)
1	3.35 (0.12)	3.40 (0.16)	3.37 ab (0.04)
3	3.57 (0.25)	3.28 (0.16)	3.42 ab (0.21)
5	3.59 (0.51)	3.62 (0.52)	3.61 bcd (0.02)
7	4.00 (0.29)	3.67 (0.48)	3.83 d (0.23)
9	3.91 (0.70)	3.36 (0.06)	3.64 bcd (0.39)
11	4.06 (0.82)	3.38 (0.21)	3.72 cd (0.48)
13	3.65 (0.22)	3.47 (0.10)	3.56 bc (0.13)
MAP effect[4]	3.67 b (0.30)	3.44 a (0.14)	

1 MAP 1: 84.5% N_2 + 15.5% CO_2
2 Mean values in column with a different letter are significantly different ($p \leq 0.05$)
3 Standard deviations are given in brackets
4 Mean values in rows with a different letter are significantly different ($p \leq 0.05$)

Analysis of variance for *C. sporogenes* (log_{10} cfu/g) in the RTE meal stored at 5°C for 7 d

Source of variation	DF	Mean square	F	P
Day	7	0.582	4.152	0.000
MAP	1	1.739	12.406	0.000
Day * MAP	7	0.313	2.236	0.036
Residual	112	0.140		

DF = degrees of freedom, F = F-test, P = significance level

Table 11 Validation of the effects of the combination of MAP and irradiation at 11.52 kGy on the inactivation and proliferation of C. sp*orogenes* (log_{10} cfu/g) inoculated into a RTE meal stored at 37°C for 13 d

Time of storage at 37°C (days)	Irradiation	Irradiation + MAP 1[1]	Time effect[2]
0	3.17 (0.18)[3]	3.10 (0.10)	3.14 a (0.05)
1	3.35 (0.10)	3.65 (0.10)	3.50 b (0.21)
3	5.59 (0.16)	5.61 (0.14)	5.60 c (0.01)
5	6.95 (0.43)	6.99 (0.49)	6.97 d (0.03)
7	7.15 (0.46)	7.10 (0.11)	7.13 d (0.04)
9	7.22 (0.35)	7.46 (0.21)	7.34 e (0.17)
11	7.74 (0.23)	7.42 (0.23)	7.58 f (0.23)
13	7.65 (0.09)	7.14 (0.13)	7.40 ef (0.36)
MAP effect[4]	6.10 a (1.87)	6.06 a (1.76)	

[1] MAP 1: 84.5% N_2 + 15.5% CO_2
[2] Mean values in column with a different letter are significantly different ($p \leq 0.05$)
[3] Standard deviations are given in brackets
[4] Mean values in rows with a different letter are significantly different ($p \leq 0.05$)

Analysis of variance for *C. sporogenes* (log_{10} cfu/g) in a RTE meal stored at 37°C for 7 d

Source of variation	DF	Mean square	F	P
Day	7	52.553	798.518	0.000
MAP	1	0.053	0.809	0.370
Day * MAP	7	0.283	4.300	0.000
Residual	112	0.066		

DF = degrees of freedom, F = F-test, P = significance level

At 5^{o}C (Table 10 and Figure 10), *C. sporogenes* counts remained more or less constant at between 10^3 and 10^4 cfu/g during the 13 d storage period. Overall, MAP did not seem to have an effect on the proliferation rate of *C. sporogenes* inoculated into the RTE meal. Statistical analysis of the data shows otherwise, MAP caused reduction in the proliferation of *C. sporogenes* counts beyond 5 d of storage. A significant interaction was observed between storage time and MAP.

Storage at 37 °C had a significant effect on the rate of proliferation of *C. sporogenes* in the RTE meal, with counts increasing over time (Table 11). At 37^{o}C, *C. sporogenes* counts increased by approximately 4 $\log_{10}$ cycles by day 5 of storage and then remained almost constant during the last 8 d of storage. MAP did not have a significant effect on the proliferation of *C. sporogenes* inoculated into the RTE meal during the storage period. A significant interaction was observed between MAP and storage time (days) at 37 °C.

Storage temperature had a major effect on the proliferation of *C. sporogenes* inoculated into the RTE meal (Figure 10). The higher the temperature (37^{o}C), the higher the growth rate of *C. sporogenes* inoculated into the RTE meal, and the lower the temperature (5^{o}C), the lower the growth rate of *C. sporogenes* inoculated into the RTE meal. Depending on the storage temperature, the longer the storage time the higher the *C. sporogenes* count inoculated into the RTE meal.

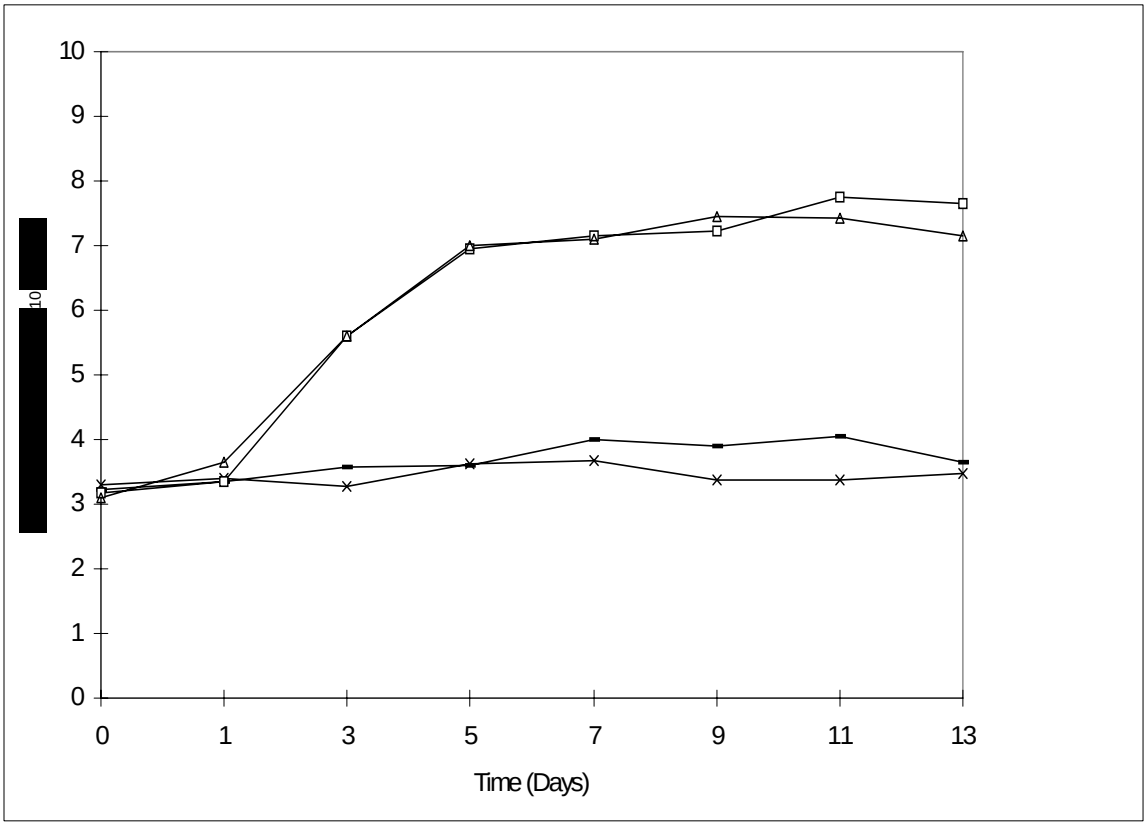

Figure 10 Validation of the effects of the combination of irradiation and MAP on the inactivation and proliferation of *C. sporogenes* (log_{10} cfu/g) inoculated into the RTE sorghum porridge and spinach (morôgo) meal over a storage period of 7 d at both 5°C and 37°C

□: Irradiation at 10 kGy, MAP 0 (air), and storage at 37°C (Irradiation)

Δ: Combination of irradiation at 10 kGy, MAP 1 (84.5% N_2 & 15.5% CO_2), and storage at 37°C (MAP + Irradiation)

–: Irradiation at 10 kGy, MAP 0 (air), and storage at 5°C (Irradiation)

×: Combination of irradiation at 10 kGy, MAP 1 (84.5% N_2 & 15.5% CO_2), and storage at 5°C (MAP + Irradiation)

CHAPTER 5
DISCUSSION

The different pre-processing and processing conditions were chosen for use in this research project for the following reasons:

- Chlorine at a concentration of 250 mg/l was used in order to reduce initial microbial counts (TPC) as well as spores (Sperber, 1982, Banwart, 1989) on raw spinach as it could potentially contain high amounts of micro-organisms due to the fact that it normally grows near the soil;
- Blanching in several changes of water was done in an attempt to further reduce TPC and inactivate enzymes and any anti-nutritional factors present in the spinach (Fennema, 1975; Potter, 1986). The decrease in total solids with blanching in more than two changes of water resulted in the use of blanching in only two changes of water in subsequent experiments;
- *Clostridium sporogenes* was used as the inoculum in this experiment because it is culturally indistinguishable from proteolytic *C. botulinum* types A, B and E strains (Sperber, 1982) which are of major concern in the food industry, but is not a pathogen;
- MAP 1 (84.5% N_2 + 15.5% CO_2) was chosen because of the high CO_2 concentrations which could potentially inhibit proliferation of aerobic microbes (Banwart, 1989). In addition, high CO_2 concentration could enhance germination of spores of facultative and obligate anaerobes (e.g. *C. botulinum*) so they could be more easily inactivated by subsequent irradiation processing;
- MAP 2 (82.3% N_2, 15.9% CO_2 + 1.8% O_2) was chosen because of the presence of O_2 which could inhibit the proliferation of potentially dangerous obligate anaerobes (pathogens) (Sperber, 1982; Banwart, 1989) during storage of the RTE meal;
- Irradiation doses up to 10 kGy were chosen as this was the highest irradiation dose recommended by the International Consultative Group on Food Irradiation (1987) for food processing, at the time the experimental design was drawn up;

- Storage temperatures of 5^{o}C and 37^{o}C were used in an attempt to simulate storage under proper cold chain storage conditions, and severe temperature abuse conditions respectively. Storage at 37^{o}C was also chosen to simulate possible storage conditions in the rural and peri-urban areas (South African townships and informal settlements) where climatic conditions are predominantly sub-tropical and refrigerated storage is not readily available.

- Preliminary experiments were carried out to optimise pre-processing parameters used in Phase 1 and 2. Phase 1 was carried out to optimise combination processing parameters used in Phase 2 for maximum possible extension of the microbiological shelf life of the RTE meal.

5.1 Preliminary experiments

There were no detectable levels (< 10 cfu/g) of aerobic mesophilic spore-formers in the spinach before and after the Cl_2 wash. This unexpected result could be due to the fact that the raw material (spinach) used was cultivated hydroponically in an enclosed area, and not contaminated by soil- or wind-borne spores, or that aerial contamination was very low and rinsing the product prior to shipment washed off any spores present on the spinach.

In comparison to the control, the reduction in microbial count (TPC) with the use of the Cl_2 wash was 99.92%. Similar results were obtained by Bloomfield & Miles (1979); Adams, *et al*. (1989); Garg, Churey & Splittstoesser (1990) and Beuchat (1997), who reported a significant decrease in aerobic mesophilic micro-organisms with increasing concentration of Cl_2 in prepared salads and fresh-cut vegetables.

Hypochlorites are effective at relatively low concentrations and are active against a wide range of bacteria and bacterial spores, as well as moulds, yeasts, bacteriophages and viruses (Banwart, 1989). The hypochlorites are considered to be more effective against Gram-negative than Gram-positive bacteria, and viruses are more resistant than bacteria

to the action of chlorine. These may be the reasons for a high percentage reduction in total plate counts in the Cl_2 washed spinach.

The low microbial counts resulting from the 250 mg/l chlorine wash is important since this would allow for a longer shelf-life of the final product provided there is no cross-contamination during and after processing, and storage conditions do not favour proliferation of micro-organisms that survived the process. However, although the latter is desirable, the absence of competition could allow for the proliferation of pathogenic micro-organisms that may be present and resistant to chlorine.

The fact that blanching did not reduce the microbial load of the spinach following the Cl_2 (250 mg/l) wash was unexpected as several scientists had previously reported on the effectiveness of blanching in reducing microbial load. Viorol (1972), Banwart (1989) and Madden (1992) reported the destruction of the normal microbial flora on fresh produce which undergo a blanching treatment. The raw materials and final product of these researchers could possibly have had a higher initial microbial count than the raw materials and final product in this research experiment due to different horticultural practices followed in their cultivation.

Possible reasons for blanching being seemingly ineffective after a chlorine wash, could be that micro-organisms that survived the Cl_2 wash at the concentrations used were possibly resistant to blanching at the processing time and temperature (77^oC for 6 min) (Luh & Kean, 1975) or that the inactivation of microbes that survived after the initial 99.92% reduction following the chlorine wash was insignificant or non-detectable (< 10 cfu/g) by the methods used.

5.2 Phase 1: Effects of MAP in combination with irradiation at different doses on microbial load in a RTE sorghum porridge and spinach meal

In Phase 1 the effects of irradiation at different dose levels under different modified atmosphere packaging conditions on the total plate counts (TPC) and inoculated *C. sporogenes* counts in the RTE meal were determined.

There were no detectable levels (< 10 cfu/g) of aerobic mesophilic spores in the RTE meal before and after processing. This result could be due to the fact that the raw material (spinach) used was cultivated hydroponically in an enclosed area resulting in low or no contamination by air- or soil-borne spores, and the sorghum meal used to make the porridge had no spores or very low numbers that were possibly not detected by the methods used.

Neither MAP treatments (MAP 1: 84.5% N_2 + 15.5% CO_2 and MAP 2: 82.3% N_2, 15.9% CO_2 + 1.8% O_2) had an effect on TPC in the RTE meal. This result was unexpected since it was postulated that the presence of high CO_2 concentrations would inhibit the growth of most aerobic mesophilic micro-organisms. The results disagreed with those obtained by Rizvi (1988), Labuza, Fu & Taoukis, (1992) and Crovetti, Ciapellano, Leopardi, Clemente & Testolin (1995). They found that MAP had an inhibiting effect on spoilage micro-organisms in minimally processed chilled foods. Reasons for the difference in findings may possibly be as a result of pre-treatments (250 mg/l Cl_2 wash) which reduced the TPC in the RTE meal significantly to such an extent that it nullified the effects of MAP, or the fact that MAP in this experiment was combined with irradiation which had a greater reducing effect on TPC masking any possible signs of a MAP effect.

Irradiation at 2 kGy reduced TPC in the RTE meal significantly, but there was no further significant reduction in TPC at higher irradiation dose levels (4, 6, 8 & 10 kGy). Similar results were obtained by McAteer, Grant, Patterson, Stevenson & Weatherup (1995) who reported that irradiation to dose levels of 2 to 3 kGy reduced the number of micro-

organisms present on all meal components of a RTE meal to less than 100 cfu/g. These researchers found that low-dose irradiation was effective in limiting the growth of spoilage micro-organisms as well as improving the safety of the product. The lack of any further reduction in TPC between 2 and 10 kGy may be ascribed to the fact that the RTE meal had a low initial microbial load which did not decrease past a certain point, in this case < 1 $\log_{10}$ cycle after 2 kGy irradiation dose level was applied.

It was thought that there would be a synergistic interaction between MAP and irradiation, where MAP would keep proliferation rate of TPC down before, during and immediately after irradiation processing. However, no such interactions were found.

Even though the results in this experiment showed that irradiation decreased the *C. sporogenes* count in the RTE meal with increasing dose, the rate of inactivation was lower than expected for the MAP treatments as indicated by the relatively high gamma D_{10}-values. Because of this low rate of inactivation, it was postulated that the interruptions during the irradiation process and the time spent at ambient temperature by the RTE meal samples after irradiation processing but before microbiological analysis, could have been the cause of the erroneous results. Samples were irradiated to 6 kGy on the first day of irradiation processing, left overnight then irradiated to 8 and 10 kGy on the following day. Ionising irradiation causes breaks in DNA strands. Restitution can occur in most of the single strand breaks, as long as the repair process is operative (Hittelman & Pollard, 1982, according to Banwart, 1989). Within the first hour of irradiation, about 90% of the DNA breaks are rejoined (Graubman & Dikomey, 1983, according to Banwart, 1989). The interruptions in irradiation process may have been long enough for the DNA repair mechanism of the *C. sporogenes* to effect repair in parts of the DNA that were damaged leading to these erroneous results. As a result of this, Phase 1 was repeated (with regard to inoculated *C. sporogenes*) using stricter control measures.

Irradiation of samples for the validation of Phase 1 was carried out from 0 to 20 kGy with 2 kGy intervals. Irradiation reduced counts of *C. sporogenes* inoculated into the RTE meal significantly, with gamma D_{10}-values (irradiation dose required to inactivate *C.*

sporogenes population by 1 $\log_{10}$ cycle) of 2.60 kGy, 2.59 kGy and 2.58 kGy for MAP 0 (air), MAP 1 (84.5% N_2 + 15.5% CO_2) and MAP 2 (82.3% N_2, 15.9% CO_2 + 1.8% O_2) respectively. These gamma D_{10}-values would explain the reasons why there were no significant differences between the inactivation effect of the following target dose levels: 0 and 2 kGy; 2 and 4 kGy; 12 and 14 kGy and 16, 18 and 20 kGy, since the actual difference in dose levels received were less than 2.59 kGy. A gamma D_{10}-value of 2.59 kGy and a target irradiation dose of 20 kGy, resulted in a 7.7 $\log_{10}$ cycle reduction in *C. sporogenes* population inoculated into the meal.

The gamma D_{10}-values obtained were higher than those reported by Botha & Holzapfel (1988), i.e. gamma D_{10}-values of 1.6 to 2.2 kGy for *C. sporogenes* in Reinforced Clostridium broth (Merck). These lower values could possibly be attributed to differences in media in which the microbes were irradiated, where the components of the RTE meal probably protected micro-organisms from the effects of irradiation (Shamsuzzaman & Lucht, 1993). Grant & Patterson (1992) reported gamma D_{10}-values of between 2.09 and 3.61 kGy for *C. perfringens* inoculated in a chilled RTE meal packaged in O_2 impermeable polyester-polyethylene bags. Physiological differences in test microbes could have been responsible for differences in gamma D_{10}-values (Botha & Holzapfel, 1988).

MAP had no effect on the inactivation and/or proliferation of *C. sporogenes* in the RTE meal. It was expected that MAP conditions with high CO_2 and no O_2 (MAP 1: 84.5% N_2 + 15.5% CO_2) would stimulate the germination of spores and enhance the growth of *C. sporogenes* (Enfors & Molins, 1978; Foegeding & Busta 1983b) during the initial 24 h period prior to irradiation processing, and that the vegetative cells would subsequently be more easily inactivated by the irradiation process. It was also expected that MAP conditions with O_2 (MAP 2: 82.3% N_2, 15.9% CO_2 + 1.8% O_2) would inhibit the growth of *C. sporogenes* (obligate anaerobe) (Banwart, 1989) inoculated into the RTE meal. However, these expectations were not realised.

These results were in disagreement with those obtained by Holland, Barker & Wolfe (1970), and King & Gould (1971), according to Hintlian & Hotchkiss (1986) who reported the enhancement of *C. botulinum* spore germination by CO_2 in phosphate buffer solutions. Furthermore, Enfors & Molin (1978) as well as Foegeding & Busta (1983b) reported that CO_2 enhanced the germination of three strains of *C. botulinum*, in phosphate buffer solutions and peptone yeast extract broth, respectively.

Possible reason for MAP having had no effect on the proliferation rate of *C. sporogenes* could be the temperature (5°C) at which the RTE meal samples (packaged in MAP 0: control, MAP 1: 84.5% N_2 + 15.5% CO_2 and MAP 2: 82.3% N_2, 15.9% CO_2 + 1.8% O_2) were stored for 24 h prior to irradiation. At 5°C *C. sporogenes* metabolic activity (germination) (Sperber, 1982; Banwart, 1989) and hence their proliferation rate were probably reduced. Another reason why MAP was seemingly ineffective against *C. sporogenes* inoculated into the RTE meal could be because the irradiation treatment with which MAP was combined could have had a greater effect in reducing counts and proliferation of *C. sporogenes* than MAP, hence masking the effect of MAP.

5.3 Phase 2: Effect of irradiation in combination with MAP 1 on the shelf-life and safety of the RTE meal stored at both 5°C and 37°C

In Phase 2 the effects of combining irradiation at 10 kGy with MAP 1 (84.5% N_2 + 15.5% CO_2) and storage at two different temperatures (5°C and 37°C) over a period of 7 d on the inactivation and proliferation of TPC (shelf-life) and *C. sporogenes* (safety) in a sorghum porridge and spinach (morôgo) RTE meal was determined.

The irradiation dose of 10 kGy was chosen for use in this phase for two reasons:

Irradiation doses up to 10 kGy were chosen as this was the highest irradiation dose recommended by the International Consultative Group on Food Irradiation (1987) for food processing, at the time the experimental design was drawn up;

Irradiating the RTE meal to a target dose level of 10 kGy reduced inoculated *C. sporogenes* spores by approximately 4 $\log_{10}$ cycles.

MAP 1 (84.5% N_2 + 15.5% CO_2) was chosen for use in Phase 2 of the research project, as it was postulated that it would minimise the effects of oxidative rancidity in the RTE meal during storage (Urbain, 1986). It was also thought that MAP 1 (84.5% N_2 + 15.5% CO_2) would inhibit the proliferation of aerobic, mesophilic bacteria (TPC) (Banwart, 1989), thus extending shelf-life of the RTE meal during the storage period following irradiation processing.

5.3.1 Shelf-life of the RTE meal

According to Jay (1985), although universal definitions of food microbial spoilage do not exist, all perishable foods reach a state of undesirability when held long enough under conditions that permit the growth of micro-organisms. Foods are free of any signs of microbial spoilage with aerobic plate counts of < 10^4/g. The same is true for most foods that contain between 10^4 and 10^5 viable bacteria/g. With counts between 10^5 and 10^6 cfu/g, some products are in states of spoilage incipiency, while counts of 10^7 to 10^8 cfu/g generally denote off-odours and/or off-flavours.

It should be noted that the level of TPC in the RTE meal samples treated with irradiation (target dose of 10 kGy) in combination with MAP 1 (84.5% N_2 + 15.5% CO_2) during the storage period at 5^{o}C was well below the microbiological criteria for prepared vegetables of 10^5 cfu/g as proposed by Shapton & Shapton (1991) and the lowest possible counts at which food microbial spoilage occurs (10^4 cfu/g) (Jay, 1985).

If the microbiological cut-off point of 10^5 cfu/g is used to determine microbiological spoilage of this particular RTE meal, then it could be said that the shelf-life of the RTE meal in this research experiment when the RTE meal were stored at 5^{o}C was:

- 3 d for the control samples;
- at least 5 d for the MAP 1 (84.5% N_2 + 15.5% CO_2) treated samples;
- at least 7 d for the irradiation (target dose of 10 kGy);

- at least 7 d for the combination of MAP 1 (84.5% N_2 + 15.5% CO_2) and irradiation (target dose of 10 kGy) treated samples.

When the RTE meal was stored at 37^{o}C, the shelf-life was:

- less than 1 d for the control and MAP 1 (84.5% N_2 + 15.5% CO_2) treated samples;
- 3 d for the irradiation (target dose of 10 kGy) treated samples;
- at least 7 d for the MAP 1 (84.5% N_2 + 15.5% CO_2) in combination with irradiation (target dose of 10 kGy) treated samples.

When the RTE meal samples were stored at 5^{o}C, the effect of MAP on TPC was most noticeable in the unirradiated samples. TPC in the control (MAP 0) samples were higher than TPC in MAP 1 (84.5% N_2 + 15.5% CO_2) for the unirradiated samples. This could be due to the presence of a high concentration of CO_2, which has a bacteriostatic effect on aerobic micro-organisms (Banwart, 1989; Varoquaux, Alagnac, Nguyen The, & Varoquaux, 1996).

The microbial growth in the unirradiated RTE meal samples packaged in MAP 1 (84.5% N_2 + 15.5% CO_2) during storage could possibly be attributed to facultative anaerobes. MAP did not seem to have any effect on TPC in irradiated samples of the RTE meal when stored at 5^{o}C. This was probably because irradiation in conjunction with the storage temperature had a greater effect, which nullified or masked the effects of MAP.

When the RTE meal samples were stored at 37^{o}C, storage time (days), MAP and irradiation had significant effects on the inactivation and/or proliferation of TPC in the RTE meal. The rate of proliferation of TPC in the meal ultimately depended on the storage temperature, regardless of the initial effect of the MAP and irradiation treatments. As was expected, proliferation was faster at 37^{o}C than at 5^{o}C, as this happens to be around the upper limit of the optimum growth temperature range of most mesophilic aerobic bacteria (Banwart, 1989).

The implication of this finding is that severe temperature abuse of the RTE meal would lead to quick proliferation of those organisms that survived the treatments and ultimately to spoilage, i.e. a short shelf-life.

The effects of MAP on TPC in the RTE meal was most noticeable with the irradiated samples of the RTE meal stored at 37^{o}C. MAP conditions inhibited or reduced proliferation rate of TPC at 37^{o}C with counts not exceeding approximately 4 $\log_{10}$ cycles during the storage period. Possible reasons for the effectiveness of MAP reducing proliferation rate of TPC at 37^{o}C, could be the presence of high concentration of CO_2 in the mixture (MAP 1: 84.5% N_2 + 15.5% CO_2) which could have inhibited the growth of aerobic micro-organisms (Enfors & Molin, 1978).

By day 3 of storage of the unirradiated samples, TPC in the RTE meal samples packaged under MAP 0 (air) and MAP 1 (84.5% N_2 + 15.5% CO_2) conditions were the same (greater than 10^9 cfu/g). This was expected as the MAP process is non-destructive but inhibitory (Banwart 1989). At 37^{o}C which is around the upper limit of the optimum growth temperature range for mesophilic micro-organisms (Banwart, 1989), rapid proliferation would occur if there were any microbes present in the final product that were not destroyed or inactivated by the various treatments.

The interaction between MAP and irradiation was synergistic at 37^{o}C, as irradiation inactivated a high percentage of the TPC in the RTE meal and MAP kept proliferation rate of surviving TPC to a minimum. But because of the fact that the RTE meal was stored at 37^{o}C, TPC proliferated at a faster rate than when stored at 5^{o}C but did not exceed 10^4 cfu/g.

5.3.2 Safety of the RTE meal

The unexpected lack of any difference in *C. sporogenes* counts in the RTE meal samples stored at 5^{o}C and 37^{o}C led to Phase 2 (with regards to *C. sporogenes*) being repeated with stricter control of the irradiation process, hence validation of Phase 2. It was expected

that at 5°C *C. sporogenes* counts would remain more or less constant as metabolic activity of the microbes are slowed, while at 37°C counts would increase to a certain point during the storage period as metabolic activity hence growth and/or proliferation increase (Sperber, 1982, Banwart, 1989).

For the validation of Phase 2 (the effect of irradiation at 10 kGy in combination with MAP 1 (84.5% N_2 + 15.5% CO_2) and storage at 5°C and 37°C on the survival and growth of *C. sporogenes*), samples were irradiated to a target irradiation dose level of 10 kGy and stored for 13 days with samples taken and tested for microbial activity (*C. sporogenes* count) on days 0, 1, 3, 5, 7, 9, 11 & 13. As was expected, there was a significant difference in *C. sporogenes* counts in the RTE meal between samples stored at 5°C and 37°C during the 13 d storage period.

Clostridium sporogenes counts in the RTE meal samples stored at 37°C were significantly higher than counts in samples stored at 5°C by day 3 of storage. This difference could be attributed to the fact that 37°C is the optimum growth temperatures for clostridial species (Banwart 1989).

The proliferation of *C. sporogenes* (obligate anaerobe) in the samples packaged in MAP 0 (air/control) was unexpected as there was supposed to be O_2 in the package which should have inhibited their growth. This could have been as a result of the modification of the atmosphere in the package by aerobic microbes, depleting existing O_2 and producing CO_2 and other by-products which could have stimulated the germination and growth of *C. sporogenes* inoculated into the RTE meal samples (Enfors & Molin, 1978, Foegeding & Busta, 1983a).

Statistical analysis of the data obtained showed that MAP had no significant effect on the proliferation of *C. sporogenes* in the RTE meal stored at 37°C, but had a significant effect at 5°C. Modified atmosphere in samples stored at 5°C reduced the growth rate of *C. sporogenes* spores inoculated into the RTE meal after 5 d of storage. MAP conditions used in this phase contained high concentrations of CO_2 (15.5%) which should have

stimulated the germination and growth of *C. sporogenes* spores (Enfors & Molin, 1978, Foegeding & Busta, 1983a) inoculated in to the RTE meal samples. The lack of MAP effect that was observed initially (prior to day 5) was probably due the effect of the temperature (5^{o}C) at which the RTE meal was stored (Banwart, 1989).

Although no literature is available on the naturally occurring counts of *C. botulinum* in spinach, it could be said that the level of contamination would depend on the contamination level in and around the soil in which the produce was cultivated (Solomon, Kautter, Lilly & Rhodehamel, 1990). Hauschild, Aries & Hilsheimer (1975) according to Odlaug & Pflug (1978) found 15 *C. botulinum* spores per 100 g of unwashed mushrooms and 41 *C. botulinum* spores per 100 g of washed mushrooms. According to Odlaug & Pflug, (1978) the National Canners Association (1962) found 10^2 to 10^3 bacterial spores per gram of harvested tomatoes. They assumed that *C. botulinum* spores would only contribute to a fraction of the total spores present.

From the above-mentioned statements on the levels of *C. botulinum* in vegetables in general, it could be assumed that *C. botulinum* spores occurring naturally on spinach would not be more than 10^3 spores per gram. The 4 $\log_{10}$ cycle reduction in *C. sporogenes* obtained with a target irradiation dose of 10 kGy (actual dose of 11.52 kGy) would therefore render the RTE meal safe from a *C. botulinum* view point, provided that the cold chain was maintained during storage and distribution of the RTE meal, and that there were no psychrotrophic strains of *C. botulinum* in the RTE meal which survived the various processes.

5.4 Implications of preserving the sorghum porridge and spinach (morôgo) RTE meal using a combination of irradiation (10 kGy) and MAP 1 (84.5% N_2 + 15.5% CO_2) processing.

The MAP-irradiation combination treatment rendered the RTE meal stable from a microbiological point of view for at least 7 d of storage regardless of whether the product was stored under proper refrigeration conditions (5^{o}C) or had undergone temperature

abuse (37^{o}C). At abuse storage temperatures, a synergistic interaction was found between MAP and irradiation processing. Since it is difficult to maintain the cold chain in developing regions, the synergistic effect between MAP and irradiation processing can be regarded as beneficial with regards to extending the shelf-life of the RTE meal.

However, the sorghum porridge and spinach relish RTE meal is a low acid food product. This together with the fact that the meal was packaged in a full barrier film under MAP conditions favouring the absence of O_2, renders it potentially dangerous from a *C. botulinum* view-point. It is therefore crucial to establish the naturally occurring *C. botulinum* levels in this type of product as well as whether this micro-organism would be able to survive and proliferate under processing and storage conditions used.

At a storage temperature of 5^{o}C for 7 d there was no microbial activity (TPC was < 10 cfu/g). Implications of this includes a desirable extension of the shelf-life of the RTE meal treated with the combination of MAP and irradiation. On the other hand, the lack of any competition for *C. sporogenes* that survived the processes could lead to them proliferating unchecked in the event that the cold chain is broken, causing a health hazard, i.e. the meal could become toxic before it spoiled.

At a storage temperature of 37^{o}C for 7 d, TPC did not exceed 3 $\log_{10}$ cycles during the storage period for the combination treatment as compared to the control, MAP and irradiation treatments on their own. The implications of this could be a longer shelf-life of the RTE meal, and lack of TPC proliferation means no competition for *C. sporogenes* (which was unaffected by MAP) which go on proliferating unchecked in the RTE meal i.e. the meal becomes toxic before it spoiled.

At a storage temperature of 5^{o}C, the *C. sporogenes* counts in the meal remained more or less constant during the storage period, with MAP 1 (84.5% N_2 + 15.5% CO_2) reducing proliferation rate of *C. sporogenes* inoculated into the RTE meal beyond 5 d of storage. However, in the event of severe temperature abuse, coupled with lack of competition (TPC), the *C. sporogenes* would proliferate rapidly resulting in health hazards, if the RTE

meal contains strains of the organism capable of growth and toxin production at low temperatures.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Chlorine at a concentration of 250 mg/l reduced the microbial load (TPC) on spinach by approximately 3 $\log_{10}$ cycles.

Blanching of spinach after chlorine treatment did not have an effect on microbial activity (TPC). Blanching in more than two changes of water led to a drastic loss in total solids, meaning a lower nutritional content of the product.

Initially, in Phase 1, it was found that interruptions during the irradiation processing of the RTE meal led to discrepancies in gamma D_{10}-values for *C. sporogenes* between the different MAP conditions. It is postulated that the duration of these interruptions (up to 14 h) may have been long enough for the microbes to initiate repair of the damaged DNA. Stricter control measures during irradiation processing resulted in more reliable gamma D_{10}-values.

Irradiation (alone) reduced *C. sporogenes* counts and total plate counts (TPC) in the RTE meal significantly. Neither MAP (82.3% N_2 + 15.9% CO_2 + 1.8% O_2 and 84.5% N_2 + 15.5% CO_2) conditions on their own or in combination with irradiation had an effect on *C. sporogenes* counts and TPC in the RTE meal during the first 24h of storage. Gamma D_{10}-values of the RTE meal were between 2.58 kGy and 2.60 kGy indicating an effective inactivation rate by irradiation. A target dose of 10 kGy (actual dose 11.52 kGy) resulted in a 4 $\log_{10}$ cycle reduction in *C. sporogenes* counts.

Irradiation reduced initial TPC and *C. sporogenes* in the RTE meal significantly. Modified atmosphere packaging (84.5% N_2 + 15.5% CO_2) did not have an effect on the growth of *C. sporogenes* in this particular RTE meal when stored at 37^{o}C. However, at a storage temperature of 5^{o}C MAP seems to have reduced growth rate of *C. sporogenes* inoculated into the meal beyond 5 d of storage. The effect of MAP on total plate count in the RTE meal depended on the storage temperature, at 5^{o}C, growth rate (TPC) was lower

than at 37°C. MAP-irradiation combination processing is synergistic with regard to TPC in the RTE meal stored at 37 °C since irradiation inactivates a high percentage of the bacteria and MAP keeps growth of surviving bacteria to a minimum. Although this might be regarded as beneficial with regard to extending the shelf-life of the RTE meal, the elimination of *C. botulinum* and thus the safety of the meal, cannot be guaranteed.

If the microbial cut-off point of 10^5 cfu/g for TPC is used to determine spoilage of the RTE meal, then the shelf-life of the RTE meal was as follows for the following treatments:

5°C-

- Control 3 d
- -MAP Alone 5 d
- Irradiation alone At least 7 d
- MAP + Irradiation At least 7 d

37°C-

- Control Less than 1 d
- -MAP Alone Less than 1 d
- Irradiation alone 3 d
- MAP + Irradiation At least 7 d

It is possible to produce a safe sorghum porridge and spinach RTE meal with a shelf-life of at least 7 d at 5°C using a combination of MAP (84.5% N_2 + 15.5% CO_2) with irradiation at a target dose level of 10 kGy, provided the following points are adhered to:

- Low initial microbial load on the raw materials and finished product.
- No cross-contamination during processing
- Ensuring the maintenance of the cold chain throughout processing, storage distribution and final end-use.

The use of alternative hurdles to MAP (e.g. nitrites and/or a_w) in conjunction with irradiation and storage at low temperature (5°C) are recommended for the processing of

the RTE meals and other low-acid foods in order to improve shelf-life and guarantee safety.

CHAPTER 7
REFERENCES

ADAMS, M.R., HARTLEY, A.D. & COX, L.J., 1989. Factors affecting the efficacy of washing procedures used in the production of prepared salads. *Food Microbiol*. 6, 69-77.

ANELLIS, A., BERKOWITZ, D., KEMPER, D. & ROWLEY, D.B., 1972. Production of types A and B spores of *Clostridium botulinum* by the biphasic method: Effect on spore population, radiation resistance, and toxigenity. *Appl. Microbiol*. 23, 734-739.

ANELLIS, A., SHATTUCK, E., ROWLEY, D. B., ROSS, Jr. E.W., WHALEY, D.N. & DOWELL, Jr. V.R., 1975. Low-temperature irradiation of beef and methods for evaluation of a radappertization process. *Appl. Microbiol*. 30, 811-820.

BANWART, G.J., 1989. Basic Food Microbiology. 2nd edition. New York: Van Nostrand Reinhold. pp. 11-48, 505-724.

BAYLISS, C.E, WAITES, W.M. & KING, N.R., 1981. Resistance and structure of spores of *Bacillus subtilis*. *J. Appl. Bacteriol*. 50, 379-390.

BEUCHAT, L.R., 1997. Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds destined for sprout production. *Int. J. Food Microbiol*. 34, 329-333.

BEUCHAT, L.R. & BRACKETT, R.E., 1990. Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. *J. Food Sci*. 55, 755-758, 870.

BEUCHAT, L.R. & BRACKETT, R.E., 1991. Behaviour of *Listeria monocytogenes* inoculated into raw tomatoes and processed tomato products. *Appl. Environ. Microbiol*. 57, 1367-1371.

BLOOMFIELD, S.F. & MILES, G.A., 1979. The antibacterial properties of sodium dichloroisocyanurate and sodium hypochlorite formulations. *J. Appl. Bacteriol*. 46, 65-73.

BOGNAR, A., 1980. Nutritive value of chilled meals. In: Glew, G. (Ed.) Advances in Catering Technology. New York: Applied Science Publishers. pp. 387-408.

BOTHA, S.J. & HOLZAPFEL, W.H., 1988. Resistance of vegetative cells and endospores of *sporolactobacillus* to gamma-irradiation. *Int. J. Food. Microbiol* 7, 169-172.

BRACKETT, R.E., 1993a. Microbial quality. In: Shewfelt, R.L. & Prussia, S.E. (Eds.) Post Harvest Handling: A Systems Approach. New York: Academic Press Inc. pp. 125-148.

BRACKETT, R.E., 1993b. Microbiological spoilage and pathogens in minimally processed fruits and vegetables. In: Wiley, R. (Ed.) Minimally Processed Refrigerated Fruits and Vegetables. New York: Van Nostrand Rheinhold. pp. 269-312

BROCKLEHURST, T.F., ZAMAN-WONG, C.M. & LUND, B.M., 1987. A note on the microbiology of retail packs of prepared salad vegetables. *J Appl. Bacteriol*. 63, 409-415.

BRODY, A.L., 1993. Fruit and vegetables. In: Parry, R.T. (Ed.) Principles and Applications of Modified Atmosphere Packaging of Foods. New York: Blackie Academic & Professional. pp. 33-36.

BUICK, R.K. & DAMAGLOU, A.P., 1989. Effect of modified atmosphere packaging on the microbial development and visible shelf-life of a mayonnaise based vegetable salad. *J. Sci. Food Agric*. 46, 339-347.

CHURCH, I.J. & PARSONS, A.L., 1995. Modified atmosphere packaging technology: A review. *J. Sci. Food Agric*. 67, 143-152.

CROVETTI, R., CIAPELLANO, S., LEOPARDI, E., CLEMENTE, G. & TESTOLIN, G., 1995. Evaluation of some physical and sensory characteristics of Mediterranean dishes packed under modified atmosphere. *Industria Conserve*. 70, 275-282.

DANIELS, J.A., KRISHNAMURTHI, R., & RIZVI, S.S.H., 1985. A review of effects of carbon dioxide on microbial growth and food quality. *J. Food Prot*. 48, 532-537.

DESMONTS, M.H., PIERRAT, N., INGERSHEIM, A. & STRASSER, A., 1995. Combination of irradiation with other treatments for improving quality of pre cooked meals. Final report presented at the final FAO/IAEA Research Co-ordination Meeting on Irradiation in Combination with Other Processes for Improving Food Quality. Pretoria, South Africa, 28 Feb-6 Mar 1995.

DEMPSTER, J.F., HAWRYSH, Z.J., SHAND, P., LAHOLA-CHOMIAK, L. & CORLETTO, L., 1985. Effect of low-dose irradiation (radurization) on the shelf-life of beef-burgers at 3^{o}C. *J. Food Technol*. 20,145-154.

DIEHL, J.F., 1983. Effects of combination processes on the nutritive value of food. In: Combination Processes in Food Irradiation. Vienna: International Atomic Energy Agency. pp. 349-366.

DIEHL, J.F., 1990. Safety of Irradiated Foods. New York: Marcel Dekker Inc. pp. 9-40, 95-144, 181-194.

DIXON, N.M. & KELL, D.B., 1989. The inhibition by CO_2 of the growth and metabolism of micro-organisms. *J. Appl. Bacteriol*. 67, 109-136.

DUODU, K.G., 1998. Nutritional Quality of Irradiated Sorghum Porridge and Spinach Relish. Minst.Agrar (Food Processing) Dissertation. Thesis. University of Pretoria. Pretoria.

DYE, M. & MEAD, G.C., 1972. The effect of chlorine on the viability of clostridial spores. *J. Food Technol*. 7, 173-176.

EMBORG, C., 1974. Inactivation of dried bacteria and bacterial spores by means of gamma irradiation at high temperatures. *Appl. Microbiol*. 27, 830-833.

ENFORS, S.O. & MOLIN, G., 1978. The influence of high concentration of carbon dioxide on the germination of bacterial spores. *J. Appl. Bacteriol*. 45, 279-285.

FARBER, J.M., 1991. Microbiological aspects of modified atmosphere packaging technology-a review. *J. Food Prot*. 54, 58-70.

FARKAS J., 1990. Combination of irradiation with mild heat treatment. *Food Control*. 1, 223-229.

FARKAS, J., 1995. Interaction of ionizing radiation with other physical and chemical factors in improving safety and storage stability of foods. Final report presented at the final FAO/IAEA Research Co-ordination Meeting on Irradiation in Combination with Other Processes for Improving Food Quality. Pretoria, South Africa 28 Feb-6 Mar 1995.

FARKAS J., INCZE, K. & ZUKAL, E., 1973. Preservation of canned vienna sausage by combination of heat and irradiation. *Acta Alimentaria* 2, 173-179.

FELLOWS, P., 1988. Food Processing Technology: Principles and Practice. Chichester: Ellis Horwood. pp. 186-196, 201-209.

FENNEMA, O.R., 1975. Physical principles of food preservation. In: Fennema, O.R. (Ed.) Principles of Food Science. New York: Marcel Dekker Inc. pp. 14, 93-132.

FIELDING, I.M., COOK, P.E. & GRANDISON, A.S., 1994. The effect of electron beam irradiation and modified pH on the survival and recovery of *Escherichia coli*. *J. Appl. Bacteriol*. 76, 412-416.

FISHER, D.A. & PFLUG, I.J., 1977. Effect of combined heat and radiation on microbial destruction. *Appl. Microbiol*. 33, 1170-1176.

FOEGEDING, P.M., 1983. Bacterial spore resistance to chlorine compounds. *Food Technol*. 37 (11), 100-104,110.

FOEGEDING, P.M. & BUSTA, F.F., 1983a. Proposed mechanism for sensitization by hypochlorite treatment of *C. botulinum* spores. *Appl. Environ. Microbiol*. 45, 1374-1379.

FOEGEDING, P.M. & BUSTA, F.F., 1983b. Hypochlorite injury of *C. botulinum* spores alters germination responses. *Appl. Environ. Microbiol*. 45, 1360-1368.

FOEGEDING, P.M. & BUSTA, F.F., 1983c. Effect of carbon dioxide, nitrogen and hydrogen gases on germination of *Clostridium botulinum* spores. *J. Food Prot*. 46, 987-989.

FUJIKAWA, H. & OHTA, K., 1994. Patterns of bacterial destruction in solution by microwave irradiation. *J. Appl. Bacteriol*. 76, 389-394.

GARG, N., CHUREY, J.J. & SPLITTSTOESSER, D.F., 1990. Effect of processing conditions on the microflora of fresh-cut vegetables. *J. Food Prot*. 53, 701-703.

GILL, C.O. & TAN, K.H., 1980. Effect of carbon dioxide on the growth of meat spoilage bacteria. *Appl. Environ. Microbiol*. 33, 317-320.

GOULD, G.W., 1989. Introduction. In: Gould, G.W. (Ed.) Mechanism of Action of Food Preservation Procedures. New York: Elsevier Applied Sciences. pp. 1-42.

GOULD, G.W., 1995. Overview: In: Gould, G.W. (Ed.) New Methods of Food Preservation. New York: Blackie Academic and Professional. pp. xv-xix.

GOULD, G.W. & JONES, M.V., 1989. Combinations and synergistic effects. In: Gould, G.W. (Ed.) Mechanism of Action of Food Preservation Procedures. New York: Elsevier Applied Sciences. pp. 401-422.

GRANT, I.R. & PATTERSON, M.F., 1992. Sensitivity of foodborne pathogens to irradiation in the components of a chilled ready meal. *Food Microbiol*. 9, 95-103.

GRANT, I.R. & PATTERSON, M.F. 1995. Use of additional hurdles to increase microbiological safety. In: Farber, J.M. & Dodds, K.L. (Eds.) Principles of Modified Atmosphere and Sous Vide Product Packaging. Lancaster: Technomic Publishing Co., pp. 263-286.

HAMBLETON, R. & RIGBY, G. J., 1970. A study of the effect of carbon dioxide on the germination and outgrowth of spores of *Clostridium butyricum* using a slide culture technique. *J. Appl. Bacteriol*. 33, 664-673.

HINTALIAN, C.B. & HOTCHKISS, J.H., 1986. The safety of modified atmosphere packaging: A review. *Food Technol*. 40 (12), 70-76.

HINTALIAN, C.B. & HOTCHKISS, J.H., 1987. Comparative growth of spoilage and pathogenic organisms on modified atmosphere packaged cooked beef. *J. Food Prot*. 50, 218.

HOBBS, G., 1986. Ecology of food micro-organisms. *Microbial Ecol*. 12, 15-30.

HOLLAND, D. BARKER, A.N. & WOLFE, J., 1970. The effect of carbon dioxide on spore germination in some Clostridia. *J. Appl. Bacteriol*. 33, 274-276.

HURST, W.C. & SCHULER, G.A., 1992. Fresh produce processing: An industry perspective. *J. Food Prot*. 55, 824-827.

INTERNATIONAL CONSULTATIVE GROUP ON FOOD IRRADIATION, (ICGFI), 1987. Task Force Meeting on the Use of Irradiation to Ensure Hygienic Quality of Food, convened by joint FAO/IAEA/WHO. Vienna.

ICGFI, 1991. Facts About Food Irradiation, Convened by Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. IAEA: Vienna.

JAY, J.M., 1985. Microbial spoilage indicators and metabolites. In: Pierson, M.D. & Stern, N.J. (Eds.) Food-borne Micro-organisms and Their Toxins: Developing Methodology. New York: Marcel Dekker, pp. 219-241.

KADER, A.A., ZAGORY, D., & KERBEL, E.L., 1989. Modified atmosphere packaging of fruits and vegetables. *CRC- Crit. Rev. Food Sci. Technol.* 28, 1-30.

KING, A.D. Jr. & BOLIN, H.R., 1989. Physiological and microbiological storage stability of minimal processed fruits and vegetables. *Food Technol*. 132-135.

KING, A.D. JR., MICHENER, H.L.D., BAYENE, H.G., & MIHARA, K.L., 1976. Microbial studies on shelf-life of cabbage and coleslaw. *Appl. Environ. Microbiol*. 31, 404-407.

KISS, I., RHEE, C.D., GRECZ, N., ROBERTS, T.A., & FARKAS, J., 1978. Relationship between radiation resistance and salt sensitivity of spores of five strains of *Clostridium botulinum* types A, B & E. *Appl. Environ. Microbiol*. 35, 533-539.

LABUZA, T.P., FU, B., & TAOUKIS, P.S., 1992. Prediction of shelf-life and safety of minimally processed CAP/MAP chilled foods: A review. *J. Food Prot*. 55, 741-751.

LAMBERT, A.D., SMITH, J.P. & DODDS, K.L., 1991. Shelf-life extension and microbiological safety of fresh meat-A review. *Food Microbiol*. 8, 267-297.

LEBEPE, S., MOLINS, R.A., CHAROEN, S.P., FARRAR IV, H. & SKOWRONSKI, R.P., 1990. Changes in the microflora and other characteristics of vacuum-packaged pork loins irradiated at 3.0 kGy. *J. Food Sci*. 55, 918-924.

LEVY, R.V., CHEETHAM, R.D., DAVIS, J., WINER, G., & HART, F.L., 1984. Novel methods for studying the public health significance of macroinvertebrates occurring in potable water. *Appl. Environ. Microbiol*. 47, 889-894.

LEISTNER, L., 1995. The applications of hurdle technology. In: Gould G. W. (Ed.) New Methods of Food Preservation. New York: Blackie Academic & Professional, pp. 1-21.

LEISTNER, L. & GORRIS, L.G.M., 1995. Food preservation by hurdle technology. *Trends Food Sci. Technol.* 6 (2), 41-46.

LICCIARDELLO, J.J., 1964. Effect of temperature radiosensitivity of *Salmonella typhimurium*. *J. Food Sci*. 29, 469-474.

LUH, B.S. & KEAN, C.E. 1975. Canning of vegetables. In: Luh, B.S. and Woodroof, J. G. (Eds.) Commercial Vegetable Processing. Westport, Conn: AVI Publishing. pp. 256-259.

MADDEN, J.M., 1992. Microbial pathogens in fresh produce--the regulatory perspective. *J. Food Prot*. 55, 821-823.

MANVELL, P.M. & ACKLAND, M.R., 1986. Rapid detection of microbial growth in vegetable salads at chill and abuse temperatures. *Food Microbiol*. 3, 59-65.

McATEER, N.J., GRANT, I.R., PATTERSON, M.F., STEVENSON, M.H., & WEATHERUP, S.T.C., 1995. Effect of irradiation and chilled storage on the microbiological and sensory quality of a ready meal. *Int. J. Food Sci. Technol*. 30, 757-771.

MINNAAR, A., TAYLOR, J.R.N. & McGILL, A.E.J., 1995. Heat irradiation combination processing as an effective method of producing high quality shelf-stable, low-acid food products. *Food Control* 6, 165-170.

MITSUDA, H., NAKAJIMA, K., MIZUNO, H. & KAWAI, F., 1980. Use of sodium chloride and modified atmosphere packaging for extending shelf-life of fish fillets. *J. Food Sci*. 45, 661-666.

MOHAMMED, N. & EHTESCHAMUDDIN, A.F.M., 1973. Optimum blanching time and effect of blanching and sulphiting on the vitamin C content of dehydrated vegetables. *Pak. J. Sci. Res.* 25 (1/2), 157-161.

MONK, J.D., BEUCHAT, L.R. & DOYLE, M.P., 1995. Irradiation inactivation of food-borne micro-organisms. *J. Food Prot.* 58, 197-208.

MOSELEY, B.E.B., 1989. Ionizing radiation. In: Gould, G.W. (Ed.) Mechanism of Action of Food Preservation Procedures. New York: Elsevier Applied Sciences. pp. 43-70.

MUFTUGIL, N., 1985. The peroxidase enzyme activity of some vegetables and its resistance to heat. *J. Sci. Food Agric*. 36, 877-880.

MURRAY, D.R., 1990. History of Food Irradiation. In: Nutman, P.S. (Ed.). Biology of Food Irradiation. Taunton: Research Studies Press. pp. 23-58.

NEELIMA & SPLITTSTOESSER., 1990. Effect of processing condition on the microflora of fresh-cut vegetables. *J. Food Prot*. 53, 701-703.

NIELSEN, S. S., 1994. Chemical Analysis of Foods. Boston: Jones and Bertlett Publishers pp. 96-99.

ODLAUG, T.E. & PFLUG, I.J., 1978. *C. botulinum* and acid foods. *J. Food Prot*. 41, 566-573.

PALLAS, J.E. & HAMDY, M.K., 1976. Effects of thermoradiation on bacteria. *Appl. Environ. Microbiol*. 32, 250-256.

PARRY, R.T., 1993. Principles and Application of Modified Atmosphere Packaging. New York: Blackie Academic and Professional. pp. 7-19.

PATTERSON, M.F., DAMOGLOU, A.P. & BUICK, R.K., 1993. Effects of irradiation dose and storage temperature on the growth of *Listeria monocytogenes* on poultry meat. *Food Microbiol*. 10, 197-203.

PATTERSON, M.F., STEVENSON, M.H., GRANT, I.R., McATEER, N.J. & STEWART, E.M., 1995. Effect of gamma irradiation on the microbiological and organoleptic quality of ready prepared chilled meals. In: Final report presented at the final FAO/IAEA Research Co-ordination Meeting on Irradiation in Combination with Other Processes for Improving Food Quality. Pretoria, South Africa 28 Feb-6 Mar 1995.

PAUL, P., VENUGOPAL, V & NAIR, P.M., 1990. Shelf-life enhancement of lamb meat under refrigeration by gamma irradiation. *J. Food Sci*. 55, 865-866.

POTTER, N.N., 1986. Food Science. 4th edition. New York: Van Nostrand Reinhold. pp. 169-245, 303-327, 579-614.

POULSEN, K.P., 1986. Optimization of vegetable blanching. *Food Technol*. 40(6), 122-129.

POWRIE, W.D. & SKURA, B.J., 1991. Modified atmosphere packaging of fruits and vegetables. In: Ooraikul, B. & Stiles, M.E. (Eds.). Modified Atmosphere Packaging of Foods. New York: Ellis Horwood. pp. 169-245.

RIZVI, S.S., 1988. Controlled and modified atmosphere packaging of fruits and vegetables. *New York's Food and Lifescience Quarterly*. 18, 19-23.

ROBERTS, T.A., 1970. Recovering spores damaged by heat, ionizing radiation and ethylene oxide. *J. Appl. Bacteriol*. 33, 74-90.

RODE, L.J. & WILLIAMS, M.G., 1966. Utility of sodium hypochlorite for ultrastructure of bacterial spore integuments. *J. Bacteriol*. 92, 1772-1775.

SANDER, E.H. & SOO, H.M., 1978. Increasing shelf-life by carbon dioxide treatment and low temperature storage of bulk pack fresh chicken packaged in nylon/surlyn film. *J. Food Sci*. 43, 1519-1523.

SATIN, M., 1993. Food Irradiation: A Guide Book. Pennsylvania: Technomis Publishing Company. pp. 1-27.

SENTER, S.D., BAILEY, J.S. & COX, N.A., 1987. Aerobic microflora of commercially harvested, transported and cryogenically processed collards (*Brassica olearacea*). *J. Food Sci*. 52, 1020-1021.

SCHUBERT, J., 1974. Irradiation of food and food constituents: Chemical and hygienic consequences. In: IAEA. Improvement of Food Quality by Irradiation. Vienna, Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, pp. 1-38.

SHAMSUZZAMAN, K., 1987. Effects of combined heat and irradiation on the survival of *C. sporogenes*. In: Proceedings of the 6th International Meeting on Radiation Processing. Ottawa, Canada.

SHAMSUZZAMAN, K. & LUCHT, L., 1993. Resistance of *C. sporogenes* spores to radiation and heat in various non-aqueous suspension media. *J. Food Prot*. 56,10-12.

SHAPTON, D.A. & SHAPTON, N.F., 1991. Safe Processing of Foods. London: Butterworth-Heinemann. pp. 392-440.

SOLOMON, H.M., KAUTTER, D.A., LILLY, T. & RHODEHAMEL, E.J., 1990. Outgrowth of *Clostridium botulinum* in shredded cabbage at room temperature under a modified atmosphere. *J. Food Prot*. 53, 831-833.

SPECK, M.E., 1984. Compendium of the Microbiological Examination of Foods. Washington D.C.: American Public Health Association.

SPERBER, W.H., 1982. Requirements of *C. botulinum* for growth and toxin production. *Food Technol*. 36 (12), 89-94.

THAYER, D.W., 1993. Extending shelf-life of poultry and red meat by irradiation processing. *J. Food Prot*. 56, 831-833.

TORRIANI, S. & MASSA, S., 1994. Bacteriological survey on ready-to-use sliced carrots. *Food Microbiol*. 27, 487-490.

URBAIN, W.M., 1986. Food irradiation. New York: Academic Press. pp. 1-22, 83-115, 170-215, 257-263.

VAN EEDEN, T.S. & GERICKE, G. J., 1996. Effect of acculturation on habitual food intake and dietary patterns of rural and urban black home economics students. *S. Afr. J. Food Sci. Nutr*. 8, 85-94.

VAROQUAUX, P., ALBAGNAC, G., NGUYEN THE, C. & VAROQUAUX, F., 1996. Modified atmosphere packaging of fresh beansprouts. *J. Sci. Food Agric*. 70, 224-230.

VAS, K., 1981. Technological feasibility of combination treatments. In: Combination Processes in Food Irradiation. Vienna: International Atomic Energy Agency. pp. 125-130.

VIOROL, F., 1972. The blanching of vegetables and fruits. *Food Process. Ind*. 41 (4), 27, 29, 31, 33.

WILKINSON, D.J., 1990. Cook-chill in perspective. *Brit. Food J*. 92, 37-41.

WILLIAMS, D.C., LIM, M.H., CHEN, A.O., PANGBORN, R.M. & WHITAKER, J.R., 1986. Blanching of vegetables for freezing: Which indicator enzyme to use. *Food Technol*. 40 (6), 130-140.

WOLFE, S.K., 1980. Use of CO and CO_2 enriched atmosphere for meats, fish and produce. *Food Technol*. 34 (3) 55-58, 63.

WORLD HEALTH ORGANIZATION (WHO), 1988. Food Irradiation: A Technique for Preserving and Improving the Safety of Food. Geneva: World Health Organization. pp. 18-43.

WORLD HEALTH ORGANIZATION (WHO), 1997. Food Irradiation – Sky's the Limit. Press Release WHO/68 19th Sept. 1997. Geneva.

WYATT, L.R & WAITES, W.M., 1975. The effect of chlorine on spores of *C. bifermentans, B. subtilis* and *B. cereus*. *J. Gen. Microbiol*. 89, 337-344.

YOUNG, L.L., REVIERE, R.D. & COLE, A.B., 1988. Fresh red meats: A place to apply Modified Atmosphere Packaging. *Food Technol*. 42 (9), 65-66, 68-69.

ZACHARIAS, R. 1980. Chilled meals: Sensory quality. In: Glew, G. (Ed.). Advances in Catering Technology. New York: Applied Science Publishers. pp. 409-416.