



**NITRITE AND IRRADIATION PRESERVATION OF A READY-TO-EAT
SPINACH RELISH AND SORGHUM PORRIDGE MEAL**

BY

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I declare that the dissertation herewith submitted for the MSc Food Science degree at the University of Pretoria, has not been previously submitted by me for a degree at any other University

ABSTRACT

NITRITE AND IRRADIATION PRESERVATION OF A READY-TO-EAT SPINACH RELISH AND SORGHUM PORRIDGE MEAL

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In South Africa, a major part of the population is concentrated in formal and informal urban areas. Most of these people depend on commercially produced food products. It has been thought that there are significant numbers of people (in urban areas) among the black societies who prefer traditional meals. However, traditional foods (e.g. spinach morôgo and sorghum porridge) are labourious to prepare, have a limited shelf life and are hardly produced commercially.

These low acid foods can be contaminated with *C. botulinum* spores. However, sodium nitrite is effective in preventing growth of germinated spores in temperature abused meals, whereas irradiation can inactivate spores of pathogenic and spoilage microbes; depending on the dose applied. A combination of these treatments therefore has the potential to improve the safety and shelf life of traditional foods.

The effect of irradiation (0, 10, 20 and 30 kGy) on the consumer acceptability of a ready-to-eat (RTE) meal consisting of spinach morôgo and sorghum

porridge was investigated. The two components of the meal remained acceptable up to a dose of 10 kGy. The limiting factor for using higher doses was the porridge component, which experienced changes in appearance, taste, and texture. Therefore the use of irradiation at 10 kGy in combination with different levels of sodium nitrite was proposed.

The preliminary processing procedures were carried out to determine the effect of chlorine wash (250 mg.l⁻¹ NaOCl) and blanching (77 °C for 6 min) in two water changes on the spinach, and cooking (mild heat treatment) on the spinach and sorghum porridge.

Chlorine wash reduced the *C. sporogenes* counts in spinach by about 1.55 log cycles, probably because of the sporocidal effect of chlorine. Blanching of spinach following the chlorine wash had no significant decrease in the spore counts. However, the clostridia count was about 1 log cycle higher after cooking the spinach, probably due to spore activation by heat during cooking. On the other hand, cooking significantly reduced the counts in the porridge by about 1.7 log cycles probably because the vegetative cells that have germinated during the cooking process were inactivated by the long exposure to heat.

The main experiment was carried out to investigate the effect of sodium nitrite at different levels (0, 50, 100, 150 and 200 mg.kg⁻¹), and the effect of a combination process on the survival of the inoculated *C. sporogenes* spores (10⁴-10⁵ spores/g) and subsequent growth of the vegetative cells. The effect of cooking alone and cooking and irradiation on the residual nitrite levels was also studied.

In both components of the meal, there was a significant decrease in the clostridia counts with increased sodium nitrite levels, probably because nitrite inhibited the vegetative cells by interfering with their metabolic systems.

However, the counts increased in the sorghum porridge component after 12 d of storage at 10 °C.

Cooking alone significantly reduced the final nitrite levels in both components of the meal, probably because of leaching during the cooking process and the possibility that nitrite might have changed to another form and evaporated with water. However, irradiation after cooking significantly increased the nitrite levels in spinach relish component probably because of the oxidation of nitrogen from sources such as protein and nucleic acids. Depending on irradiation dose, higher levels of nitrite were produced in samples that received a relatively higher dose.

In both components of the meal, nitrite in combination with irradiation reduced the *C. spogenes* counts to less than 10 cfu/g. This could be due to the low initial counts after cooking and the higher sensitivity of the vegetative cells compared to that of the spores. However, growth was unexpectedly observed in the spinach relish component after day 6 and 12 in samples treated with 0, and 150 and 200 mg.kg⁻¹ sodium nitrite respectively and then irradiated at 10 kGy.

A safe RTE meal could be expected when a pre-processing, followed by a combination treatment of at least 50 mg.kg⁻¹ sodium nitrite and a target dose of 10 kGy was applied, provided there is no post contamination of the meal.

However, the use of higher levels of sodium nitrite in combination with irradiation at 10 kGy are recommended for processing spinach relish and sorghum porridge meal components in order to produce a safe ready-to-eat meal with a longer shelf life.

UITTREKSEL

PRESERVERING VAN 'N EETGEREED SORGHUMPAP EN SPINASIEGEREG DEUR NITRIET EN BESTRALING

DEUR

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In Suid-Afrika is 'n groot deel van die bevolking saamgetrek in formele en informele stedelike gebiede. Hierdie mense is grootliks afhanklik van kommersieel-geproduseerde voedselprodukte. Na verwagting is daar groot getalle swartmense in stedelike gemeenskappe wat tradisionele voedsel verkies. Baie van die tradisionele voedsels (bv morog/spinasie en sorghumpap) is tydrowend om te berei, het 'n beperkte goedhouvermoë en is moeilik om op kommersiële skaal te berei.

Hierdie voedsels het 'n lae suurheid en kan gekontamineer word met spore van *Clostridium botulinum*. Natriumnitriet kan egter die groei van ontkiemde spore in temperatuurmishandelde voedsel verhoed, terwyl bestraling die spore van patogene en bederwende bakterieë kan inaktiveer, afhangende van die aangewende dosis. 'n Kombinasie van hierdie behandelings kan dus moontlik die veiligheid en goedhouvermoë van tradisionele voedsels verbeter.

Die invloed van bestraling (0, 10, 20 en 30 kGy) op die verbruikersaanvaarbaarheid van 'n eetgereed maaltyd bestaande uit morog (spinassie) en sorghumpap is ondersoek. Die twee komponente van die maaltyd was aanvaarbaar tot by 'n bestralingdosis van 10 kGy. Die beperkende faktor vir die gebruik van hoër dosisse was die pap-komponent wat veranderings in voorkoms, smaak en tekstuur ondergaan het. Gevolglik word die gebruik van bestraling teen 10 kGy in kombinasie met verskillende konsentrasies natriumnitriet voorgestel.

Die effek van voorafprosessering in die vorm van spoel in 'n chlooroplossing ($250 \text{ mg.l}^{-1} \text{ NaOCl}$) en blansjering ($77 \text{ }^\circ\text{C}$ vir 6 min) in twee watervervangings, en kook (matige hittebehandeling) op die spinassie en sorghumpap is ondersoek.

Spoel in chlooroplossing het die aantal *Clostridium sporogenes* met ongeveer 1.55 log-siklusse verlaag, waarskynlik as gevolg van die spoordodende effek van chloor. Blansjering van die spinassie na die spoel in chlooroplossing het geen betekenisvolle afname in spoortelling tot gevolg gehad nie. Die klostridiatellings van die spinassie was egter 1 log-siklus hoër na kook, waarskynlik deurdat die spore geaktiveer is deur die hittebehandeling gedurende die kookproses. Daarenteen het die kookproses die tellings in die pap met ongeveer 1.7 log-siklusse verlaag, waarskynlik omdat die spore gedurende die langdurige verhitting ontkiem het en die vegetatiewe selle toe deur die hoë temperatuur gedood is.

Die hoof eksperiment was daarop gemik om die effek van natriumnitriet teen verskillende konsentrasies (0, 50, 100, 150 en 200 mg.l^{-1}), en die effek van 'n kombinasiebehandeling op die oorlewing van die bygevoegde *C. sporogenes*-spore (ongeveer 10^5 spore/g) en groei van die vegetatiewe selle te bestudeer. Die effek van kook alleen en van kook en bestraling gekombineer, is ook bestudeer.

In beide komponente van die maaltyd was daar 'n betekenisvolle afname in klostridiatellings namate die nitrietkonsentrasie toegeneem het, waarskynlik weens inhibering van die vegetatiewe selle. In die sorghumpap het die tellings egter toegeneem gedurende 12 dae opberging by 10 °C.

Kook alleen het die finale nitrietkonsentrasies in beide komponente van die maaltyd betekenisvol verlaag, waarskynlik weens uitloging tydens die kookproses en die moontlikheid dat die nitriet omgesit is na 'n ander produk wat saam met die water verdamp het. Bestraling na die kookproses het die nitrietvlakke in die spinasie egter betekenisvol verhoog, waarskynlik weens die oksidasie van die stikstof aanwesig in proteïene en nukleïensure. Hoër vlakke van nitriet is verkry in monsters wat aan hoër bestralingsdosisse blootgestel is.

In beide komponente van die maaltyd, het nitriet in kombinasie met bestraling die tellings van *C. sporogenes* verlaag na benede 10 kve/g. Dit mag die gevolg wees van die lae tellings na die kookproses en die groter gevoeligheid van die vegetatiewe selle in vergelyking met die spore. Teen die verwagting in het bakteriegroei in die spinasie voorgekom na 6 en 12 dae in monsters wat met 0 mg.kg⁻¹ natriumnitriet behandel is en in monsters wat bestraal en met respektiewelik 150 en 200 mg.kg⁻¹ natriumnitriet behandel is.

'n Veilige eetgereed voedsel kan verwag word as 'n voorafprosessering toegepas en gevolg word deur 'n kombinasiebehandeling van minstens 50 mg.kg⁻¹ natriumnitriet en bestraling teen 'n mikpunt dosis van 10 kGy, mits die voedsel nie daarna gekontamineer raak nie.

Die gebruik van hoër konsentrasies natriumnitriet in kombinasie met bestraling teen 10 kGy kan aanbeveel word vir die prosessering van spinasievoorgereg- en sorghumpap-maaltydkomponente ten einde 'n veilige eetgereed maaltyd met 'n langer goedhou vermoë te produseer.

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CHAPTER 1

INTRODUCTION

Sorghum cereal grain has been acknowledged to be one of the most important subsistence grains in most African societies especially in the semi-arid areas (Dendy, 1995). In Southern Africa, porridge made from sorghum has traditionally been considered to be the staple diet of many Black societies, particularly among the Tswana of Botswana and South Africa (Taylor, Dewar, Taylor & Von Ascheraden, 1997). Van Eeden & Gericke (1996) found thick porridge to be the most popular traditional food source, and wild greens, cooked pumpkin and a mix of spinach, cabbage and turnips were the vegetables used on a regular basis.

In South Africa, about 60% of the population are concentrated in formal and informal urban areas. Most of these people depend on commercially produced food products. It has been thought that there are significant numbers of people (in urban areas) among the black societies who prefer traditional meals. One example of these meals is spinach (morôgo) or amaranthus based relishes which is consumed with either maize or sorghum porridge. These traditional foods have a short shelf life, are laborious to prepare (Duodu, Minnaar & Taylor, 1999) and are hardly produced commercially (Obilana, 1998).

Survival and growth of spores of pathogenic micro-organisms in these traditional foods can be of safety concern especially as most leafy vegetables as well as cereal porridges are low acid foods (pH > 4.6). The spores of mesophilic anaerobic bacteria germinate and grow readily in low acid foods (Banwart, 1989).

According to Obilana (1998), the survival and growth of pathogens such as *C. butulinum* were possible in a ready-to-eat spinach and sorghum porridge meal

produced using a combination of MAP (84.5% N₂ + 15.5% CO₂) with irradiation dose level of 10 kGy if the meal was temperature abused. Hence, there is a need to try other methods or a combination of methods to ensure the safety of low acid food products. Another combination process that could be used is sodium nitrite in combination with irradiation.

Food irradiation is among the major new food processing and preservation techniques, which has received much attention due to its potential effect on the microbiological safety of food. From the microbiological point of view, food irradiation has two potential advantages: (1) it reduces the number of pathogens and so increases food safety, (2) it reduces the number of spoilage organisms and therefore extends the shelf life of the product (Institute of Food Technology, 1983).

Sodium nitrite has been found to be effective in preventing outgrowth of *Clostridium botulinum* in temperature abused cured meat products. A considerable amount of research has been done relating to the use of nitrites in perishable cured meat such as bacon, sausages, vieners and canned hams (Christiansen, 1980) but very little research has been done on ready-to-eat (RTE) meals. For the most part, these meals are given a mild heat treatment (71.1^oC or less) and with few exceptions, they must be held refrigerated to achieve their expected shelf life. If refrigerated storage could be assured, there would be no potential botulinal hazard in food products (Christiansen, 1980; Austin, Dodds, Blanchfield & Farber, 1998).

CHAPTER 2

LITERATURE REVIEW

According to Patterson, Stevenson, Grant, McAteer & Stewart (1998) there is an increasing consumer demand for high quality, convenient, and fresh-like nutritious foods that are cooked and stored chilled (0-3⁰C) and reheated prior to consumption. These easy-to-prepare foods have led to the production of minimally produced foods, usually referred to as ready-to-eat (RTE) foods.

2.1 Ready-to-eat (RTE) foods

According to Henry (1993), ready-to-eat (RTE) foods may be broadly defined as foods that have undergone major processing by the manufacturer such that they require little or no secondary processing or cooking before consumption. This means that apart from heating or rehydration, the food is ready to eat. A food may be classified as RTE food if it meets the following criteria:

- The food must have undergone a considerable amount of food preparation by the manufacturer before it reaches the retailer.
- The food produced must require minimal cooking or processing by the consumer before consumption
- The preparation time required before consumption by the consumer must be minimal (Henry, 1993).

Consumption of RTE foods that are held after preparation has raised concern among food microbiologists because of the occurrence of several disease causing bacteria such as *C. botulinum* that can grow at 5 ⁰C. Therefore, if the food is not properly treated during initial processing, surviving organisms will slowly multiply at normal refrigeration temperatures

over extended storage times and produce toxin slowly especially over a long storage time (King & Bolin, 1989).

Efforts to preserve the sensory and microbiological quality of cooked, chilled meals have centered on the use of vacuum packaging and modified atmosphere packaging (Young, MacFie & Light, 1989). However, an alternative approach could be the use of low irradiation doses to extend the shelf life of chilled ready-to-eat meals and reduce the risk of food poisoning (McAteer, Grant, Patterson, Stevenson & Weatherup, 1995).

Although extensive research has been carried out to assess the microbiological and organoleptic effects of irradiating individual food items, little research has been reported on the irradiation of cooked RTE meals. In general, the irradiation of cooked foods has not been studied extensively because it was generally assumed that the cooking process provided adequate microbiological control, and additional irradiation would be superfluous (McAteer *et al.*, 1995). However, studies have shown that cooked food which are not subject to strict temperature control during cooking and storage, or which are recontaminated after cooking, could represent a potential health risk such as botulism (Schafheitle & Light, 1989).

2.2 *Clostridium botulinum*

Clostridium botulinum is a species of anaerobic, spore forming, rod shaped bacteria, producing a protein with a characteristic neurotoxicity. Severe food poisoning (botulism) results from the consumption of botulinum toxin (botulin) produced in food in which growth of this organism has occurred (Kautter & Lynt, 1984).

Spores of this organism are widely distributed in soil and in sediments of oceans and lakes so that foods may become contaminated with *C. botulinum* from many possible sources. For example, vegetables grown in these areas are liable to contamination by the botulin spores (Elliott,

Clark, Lewis, Lundbeck, Olson & Simonsen, 1978). Inadequately processed home-canned foods such as meat and many kinds of vegetables, especially green beans, asparagus, spinach have been frequently associated with botulism (Kautter & Lynt, 1984).

Ready-to-eat meals that are cooked and stored frozen until required for consumption are well established in the food industry. These foods have not been indicated in botulism. However, it is known that the spores of *C. botulinum* may survive long storage periods in raw and precooked frozen foods and that during warm holding, after thawing, the spores are able to germinate and form toxins.

An essential objective in certain food preserving processes is to prevent the development of botulinum toxin. The methods applied must either (i) destroy all botulinum spores, (ii) prevent their germination, or (iii) provide an environment which prevents growth and toxin formation (Elliott *et al.*, 1978).

Pretreatment of vegetables with a solution containing chlorine, blanching and cooking before further processing can be used to reduce the number of contaminating microorganisms, but processes such as cutting and mixing may introduce additional contamination (Vescovo, Torriani, Orsi, Macchiarolo & Scolari, 1996), therefore care should be taken during processing to prevent post harvest contamination.

2.3 Pre-processing of vegetables

With regard to vegetables, there is an increasing market for retail packs of processed vegetables, especially mixed salad vegetables that has been sliced, chopped, or grated. These packs are intended to be maintained at a temperature between 0 °C and 8 °C in order to retard deterioration in quality and multiplication of microorganisms (Brocklehurst, Zaman-Wong & Lund, 1987).

Several studies on vegetables have shown that the numbers of bacteria present on fresh vegetables at harvest can be in the region of 10^5 to 10^6 /g. The initial microbial load of these products depends on the origin of the vegetables, on agricultural practices and on conditions of harvesting and processing (Vescovo *et al.*, 1996). In addition, the number can be greatly affected by the amount of moisture present (Brocklehurst *et al.*, 1987).

Vegetables are normally mechanically harvested. Mechanical harvesters do not distinguish between the wanted vegetable material and other extraneous plant material and soil. Some of these are removed by fans and belts on the harvester before the crop is transported for further processing. Cleaning operations must be very thorough to remove the unwanted material and wash the vegetables (Brackett, 1993).

2.3.1 Washing

Vegetables harvested from underground, such as carrots and potatoes, require the removal of adhering soil and stones. Several systems are available to remove the soil, starting with dry brushing or soaking to remove the bulk of dirt, followed by washing. Washing involves wet scrubbing with rotary brushes or rubber fingers, followed by rinsing in a rod washer (Rutledge, 1991).

Leafy vegetables, such as spinach, are difficult to clean as extraneous material lodges between the leaves. Leaves are cleaned by floating them in tanks of water where the water is agitated with air or streams of water. This separates the leaves and removes soil (Rutledge, 1991).

In fact, the aim of washing is to remove field soil, surface microorganisms, fungicides, insects and pesticides. The degree to which washing reduces microbial load depends on the product in question and the procedure employed (Brackett, 1993). There are laws specifying maximum levels of these contaminating materials that may be retained in vegetables; in most cases the allowable residual level is virtually zero. Wash water-containing

detergents and other sanitizers such as chlorine can essentially completely remove these residues (Rutledge, 1991).

Chlorine or chlorine compounds have been used for food industry sanitation in several forms, including sodium hypochlorite (NaOCl), calcium hypochlorite (Ca(OCl)₂), Cl₂, chlorine dioxide (ClO₂), chlorinated trisodium phosphates, and chloramine compounds (Brackett, 1993).

Chlorine compounds are used effectively to sanitise food-processing equipment, food containers, and for washing raw food products. The use of chlorinated water in a food processing plant reduces or prevents accumulation of microorganisms. The knowledge that sublethal doses of hypochlorite may damage spores, stresses the importance of maintaining the appropriate chlorine concentrations used in food industry (Foegeding, 1983).

Different researchers have reported the effect of concentration of chlorine compounds, exposure time, and exposure temperature on inactivation of spores. Increased exposure time, elevated temperature, and higher chlorine-compound concentration, all favour sporicidal activity (Foegeding, 1983). According to Obilana (1998), washing with chlorinated water (250 mg/l) significantly reduced the initial microbial load of spinach (3 log₁₀ cycles). This was probably because hypochlorites are effective at relatively low concentrations and active against a wide range of bacteria and bacterial spores (Obilana, 1998).

2.3.1.1 Mechanism of sporicidal activity

According to Foegeding (1983), Rode & Williams (1966) found that cell walls of Gram-positive bacteria and spores of *Bacillus megaterium* were dissolved partially by hypochlorite. Electron microscopy studies with chlorine treated *Bacillus anthracoides* and *Bacillus cereus* indicated that chlorine initially caused separation of the spore coats from the cortexes, followed by sequential dissolution of the layers of the spores. Chlorine

removed a protein from spores of *Clostridium bifermentans*, *Bacillus cereus*, and *Bacillus subtilis* which had the same mobility by polyacrylamide gel electrophoresis as protein removed by sodium hydroxide from coats of *C. bifermentans* spores. Protein also was removed from hypochlorite-treated *C. botulinum* spores. Chlorine-treated *Bacillus* and *Clostridium* spores lost dipicolinic acid, calcium, RNA, and DNA to the surrounding medium. These results suggested that spore coats as well as underlying layers may be disrupted by chlorine, altering the permeability of spores. Spores with intact coats were not susceptible to germination by lysozyme, whereas hypochlorite treatment permitted germination of spores by lysozyme (Foegeding & Busta, 1983b).

In general, the spore coat appears to be both a 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 (Foegeding & Busta, 1983b). The potentiation of hypochlorite or chloramine sporicidal activity by other compounds such as NaOH, alcohol and NH₃ respectively, also may result from the effect of these compounds on spore-coat protein. It is possible that the coats of *Clostridium* spores are generally less tenacious and less resistant to oxidation than are the coats of *Bacillus* spores, thereby imparting less hypochlorite resistance to clostridial spores than that of *Bacillus* species (Foegeding, 1983).

Foegeding & Busta (1983a) reported that 12 to 28 µg of free available chlorine/ml reduced the rate of *C. botulinum* spore germination in some but not all media examined. It was also reported that mild chlorine treatment increased germinating mutants of *C. bifermentans*. They further suggested that the increased germination mutants can be due to the fact that these mutants may have altered coats and the faster germination rate of the spore could have resulted from increased permeability of the spores resulting from removal of coat protein. Where chlorine inactivated the

germination mechanism of spores, lysozyme inactivated germination; therefore, these spores did not generally grow out to form cells.

- Sublethal damage of spores

Pretreatment of spores with sublethal concentrations of chlorine may increase heat and chemical sensitivity of the spores (Foegeding & Busta, 1983b). Waites, King & Bayliss (1977) investigated the effect of chlorine and heat on spores of *Clostridium bifermentans*. They found that *C. bifermentans* spores treated with hypochlorite and subsequently mildly heated (80°C) lost dipicolinic acid and much of the cortex to the surrounding medium, and had swollen protoplasts. Such results show that chlorine treatment may have altered the coat and cortex, thereby increasing the susceptibility of the spores to mild heat.

It may also be possible that exposure to sublethal doses of hypochlorite may injure bacterial spores. Foegeding & Busta (1983b) reported injury of *C. botulinum* 62A and 12885A spores by exposure to 12 or 28 µg of free available chlorine/ml (2 min, 25°C, pH 7.0). Injury was manifested by differential enumeration of hypochlorite-treated spores by two recovery procedures and by reduced rates and extents (compared to non-hypochlorite-treated spores) of germination in some, but not all germination media examined.

L-alanine is effective as an organic germinating agent (germinant) for many bacterial spores. It apparently reacts at discrete sites on the bacterial spore to trigger the germination processes (Foegeding & Busta, 1983b). The rate and extent of germination (measured by phase-contrast microscopy) in 2 h of hypochlorite-injured *Clostridium botulinum* spores in a defined medium containing L-alanine as the germinant was reduced compared to non-hypochlorite-treated spores. Addition of lactate or malate to the L-alanine germination medium restored the ability of the injured spores to germinate. Neither lactate nor malate was an effective germinant on its own. Increasing the L-alanine concentration 10 to 100-fold also

restored the ability of hypochlorite-injured spores to germinate (Foegeding & Busta, 1983b). They further suggested that this L-alanine concentration was required for activity. Lactate and malate may function by activating the hypochlorite-modified or non-hypochlorite-modified L-alanine germination sites.

2.3.2 *Blanching*

Blanching is achieved by either brief immersion of foods into hot water or the use of steam. The method is used to destroy enzymic activity in vegetables and some fruits, prior to further processing. As such, it is not intended as a sole method of preservation but as a pre-treatment which is normally carried out between the preparation of the raw material and later operations (particularly heat sterilisation, dehydration and freezing). Blanching is usually combined with cleaning of food, to achieve savings in energy consumption, space, equipment costs and reduction of initial microbial load from a safety point of view (Boast, 1993).

Blanching has also other advantages depending upon the method of further processing of the vegetables. Vegetables for canning are blanched mainly to shrink the vegetable to obtain the correct fill weight in the can and to remove the gases from the intercellular spaces which would otherwise cause oxidation of the product, corrosion of the can and low can vacuum. Blanching also plays a role in enhancement or fixing of the green colour of certain vegetables, and in reduction of the numbers of microorganisms on the foods (Rutledge, 1991).

The method of blanching employed depends on the products in question, size of packs, and other related factors. When water is used, it is important that bacterial spores are not allowed to build up sufficiently to contaminate foods. To achieve adequate blanching, food is heated rapidly to a pre-set temperature, held for a pre-set time and cooled rapidly to near ambient temperatures. The factors which influence blanching time are (1) the type

of vegetable, (2) the size of the pieces of food, (3) the blanching temperature and (4) the method of heating (Fellows, 1988).

Although the primary function of blanching is not to destroy microorganisms, the amount of heat necessary to effect destruction of most food enzymes is also sufficient to reduce the number of vegetative bacterial cells significantly (Jay, 1992). Reduction of initial microbial loads as high as 99% upon blanching has been claimed. Most vegetative bacterial cells can be destroyed at milk pasteurisation temperatures (62.8⁰C for 30 min). This is especially true of most bacteria of importance in the spoilage of vegetables (Rutledge, 1991).

In fact, blanching reduces the numbers of contaminating microorganisms on the surface of the foods and hence assists in subsequent preservation operations. This is particularly important for further preservation of foods, as the time and temperature of processing are employed to achieve a specified reduction in cell numbers. If blanching is inadequate, a larger number of microorganisms are present initially. This may result in a large number of spoiled food units after processing (Fellows, 1988).

Contrary to studies by different researchers, Obilana (1998) showed that blanching (at 77⁰C for 6 min) after chlorine wash (250 mg/l NaOCl) did not reduce the microbial load of spinach significantly. He therefore suggested that the initial microbial load of the product could attribute to these differences, which could be due to different horticultural practices followed in cultivating the raw materials. Another possible reason was that microbes which survived the chlorine wash were resistant to blanching at the processing time and temperature (77⁰C for 6 min) or that the inactivation of microbes that survived after the initial reduction following the chlorine wash were insignificant or non-detectable (< 10 cfu/g) by the method used.

2.3.3 Cooking

By definition, cooking raises the temperature of the food. This results in a number of simultaneous and interrelated processes that influence the flavour, texture, appearance, nutrient content and safety of the food. As with all forms of cooking, the process is intended to improve the palatability and digestibility of the food, and making it more appetising. Unlike industrial food preparation and catering or food service, domestic cooking is carried out in the end user's home by people who may not have any technical knowledge of what is happening from an engineering or biochemical point of view (Rosenthal, 1993).

There are different techniques of cooking which reflect the way in which the temperature of the food is raised. Clearly, there are two basic ways in which energy can be applied to a foodstuff, resulting in a rise in temperature. The traditional route is by contact with a heated medium which causes heat to flow to the surface of the food and then on to the center by conduction. An alternative route is to apply electromagnetic energy (Fellows, 1988).

There are only two basic cooking methods: wet and dry. Wet cooking includes boiling, steaming, pressure-cooking, stewing, and poaching. During wet cooking, the surface temperature of the food does not exceed the boiling point of water (usually 100⁰C, but up to about 120⁰C using pressure cookers); consequently, the surface remains moist (Rosenthal, 1993).

The heat treatment applied during cooking of foods may inactivate spores. For non-proteolytic *C. botulinum* in phosphate buffer (pH 7.0) decimal reduction times at 82.2⁰C ($D_{82.2^0C}$) of less than 2 min have been reported (Stringer, Fairbairn & Peck, 1997). However, the measured heat resistance depends on many factors including the strain tested, its method of preparation and the heating and recovery medium (Kim & Foegeding, 1993 according to Stringer *et al.*, 1997). In particular, the presence of lysozyme

in the recovery medium substantially increases the recovery of heat damaged spores (Peck, Fairbairn & Lund, 1992a). It is thought that lysozyme can substitute for a heat-damaged germination system in a sub-population of lysozyme-permeable spores (Peck, Fairbairn & Lund, 1992b). The fact that spores can be sublethally damaged by heat treatment may affect their ability to germinate and grow at low temperatures during storage (Stringer *et al.*, 1997).

2.4 Food irradiation

Radiation may be defined as the emission and propagation of energy through space or through a material medium in the form of waves (Satin, 1993). The type of radiation of primary interest in food preservation is electromagnetic. In the electromagnetic spectrum, the various radiations are separated based on their wavelengths, with the shorter wavelength being the most damaging to microorganisms. The electromagnetic spectrum is further divided as follows with respect to the radiations of interest in food preservation: microwaves, ultraviolet rays, X-rays, and gamma rays (Jay, 1992).

The radiation of primary interest in food preservation are ionising radiations, which are defined as those radiations that have wavelengths of 2000 Å or less, for example, alpha particles, beta rays, gamma rays, X-rays, and cosmic rays. Their quanta contain enough energy to ionise molecules in their paths (Jay, 1992).

By definition, food irradiation is a process in which energy of high level is used to ionise a material (in this case food). Ionisation takes place because the ionising radiations (depending on the source of radiation) are able to eject electrons from molecules, thus disrupting the structure of molecules. If the molecule is DNA (deoxyribonucleic acid), which is the cell's genetic code that allows it to multiply, the consequences can render the cells unable to replicate (Murano, 1995a).

The numerous applications of food irradiation can be classified by dose level. Low dose applications (up to about 1 kGy) are used to inhibit sprouting, to control insect infestations and to delay maturity. Medium dose applications (1 to 10 kGy) are used to reduce the microbial load, prolong shelf life of products and to reduce the load of pathogens in products. High dose applications (10 to 50 kGy) are used to achieve commercial sterilisation, enabling food products to be stored at ambient temperatures with suitable packaging, such as shelf stable products (Skala, McGown & Warning, 1987).

2.4.1 Effect of food irradiation on microorganisms

Ionizing radiation is particularly efficient in its ability to kill life forms without raising the temperature of a particular food. Since they destroy microorganisms without appreciably raising the temperature, the process is termed "cold sterilisation" (Jay, 1992). Because the process does not add heat to food when applied, the nutritional degradations caused by heating are not created. However, there is some potential nutrient loss (especially vitamins), texture and flavour changes when high irradiation doses are applied to foods. For this reason, the recommended dose level in applying irradiation to certain food preservations has been 10 kGy or less (Murano, 1995a). However, WHO (1997a) reports that the food irradiation technology is safe as long as sensory qualities of food are retained and pathogens are destroyed, therefore the actual amount of ionising radiation applied is of secondary consideration. This was based on scientific evidence that food irradiation can be used effectively to eliminate spores of *C. botulinum* and all spoilage microorganisms, that it does not compromise the nutritional value of the foods, and that it does not result in any toxicological hazard (WHO, 1997b). Recognizing that, in practice, the doses applied to eliminate the biological hazards would be below those doses that might compromise sensory quality, the Study Group concluded that no upper dose limit need be imposed (WHO, 1997b).

This ability of ionising radiation to destroy life forms with a relatively low energy input and hence a relatively small amount of chemical change, is, of course, the major reason for its use in food processing. Thus, the numbers of microorganisms and insects in foods can be drastically reduced without any appreciable alteration to the taste or texture of the food (Deeble, Jabir, Parsons, Smith & Wheatley, 1990).

According to the Task Force Report (1989), it has been pointed out that a substerilising dose of ionising energy, such as might be designed to delay spoilage of food and destroy microorganisms of public health significance, does indeed leave behind organisms that are more resistant to ionising energy than the ones that were killed. These surviving microorganisms consist of spores of spore-forming bacteria and some highly resistant vegetative cells. The food so treated must be preserved subsequently by one of the traditional methods, such as refrigeration or maintenance in a dry condition, to derive the maximum benefit from the reduction in numbers of bacteria.

2.4.1.1 Mode of action

Ionising radiations are mutagenic for most bacteria and are lethal for all of them. For the vast majority of bacteria the critical target for inactivation is the chromosome, a single, circular molecule of DNA containing several million base pairs (Jay, 1992). According to Moseley (1990) the sensitivity of bacteria to ionising radiation is not solely due to results of chromosome damage because most bacterial species cannot be inactivated by similar doses of radiation although there is no great variation in the sizes of bacterial chromosomes.

On irradiating a particular system, a given "target" can be chemically altered either by the direct ionisation of molecules of the "target" (the "direct effect") or by the attack of reactive species (e.g. free radicals) formed by the ionisation and excitation of the molecules surrounding the target (the "indirect effect"). The probability of a high-energy photon interacting with a

particular atom depends on its electron density and in the biological systems where the most common atoms (C, O, N, and H) are present. These atoms are distributed more or less evenly so that initial ionisation occurs more or less randomly throughout the system (Deeble *et al.*, 1990).

The proportion of chemical bonds affected by the doses of ionising radiation applied to control insects and other living organisms in foods is relatively small. As a point of reference, fewer than ten chemical bonds in each million chemical bonds present in moist food are broken per kilogray of ionising energy absorbed. Breakage of enough bonds in the DNA, however, is fatal to all living cells (Murano, 1995a). Breakage of chemical bonds may also disrupt the cytoplasmic membrane, which maintains the integrity of living cells (Task Force Report, 1989). However, the primary mechanism by which irradiation destroys a microorganism is by breaking bonds on the DNA molecule, thereby rendering the cell unable to replicate (Murano, 1995a).

A given dose of ionising radiation may be fatal to certain cells, while only injuring other similar cells. Under favourable conditions, injured cells may undergo self-repair. The effects on an organism as a whole are the consequence of the effects on all of its parts (Task Force Report, 1989).

2.4.1.2 Microorganisms resistance to irradiation

Living organisms have the ability to repair breaks in their DNA, to metabolise unneeded chemical compounds that may be produced by doses of ionising radiation normally received, and to survive. The DNA must be "hit" many times before the cell in which it occurs is irreparably damaged. Further protection is provided by the ability of surrounding cells to digest or kill irreparably damaged cells (Task Force Report, 1989).

It could also be possible that the resistance of microorganisms to irradiation is due to the fact that most resistant bacteria have evolved enzymic mechanisms for keeping their DNA in good repair in the face of

environmental onslaught. Thus, although the chromosomes of bacteria are intrinsically very sensitive to potentially lethal damage as a result of exposure to ionising radiation, the amount of such damage gives them considerably greater resistance to such radiations than would otherwise be the case (Moseley, 1990).

The efficiency with which different bacteria repair the radiation-induced damage to their DNA varies considerably. The effects of ionising radiation upon living tissues depend upon the nature and condition of the tissues and the conditions of exposure. The content of water is a very important factor. The drier the cell, the less sensitive it is to ionising energy (Murano, 1995a). It has been established that in some instances the radiation resistance of some bacteria can be increased step-wise up to two fold by a few repeated doses at a constant dose level, and several fold by a progressive increase of applied dose (Desrosier, 1977).

Among the bacteria, those in the spore state are generally the most resistant. Bacterial spores are more resistant to the lethal action of ionising radiation than their corresponding vegetative cells by a factor of about 5 to 15 (Task Force Report, 1989). In general, microorganisms differ in their sensitivity to irradiation, depending on morphological variations, just as they differ in their sensitivity to heating, drying and freezing (Moseley, 1990).

The type of irradiation and, within limits, the pH of the food seems to have little influence on the dose needed to inactivate the microorganisms. It has been stated that the resistance of a given species of microorganisms to ionising radiations parallels in general its resistance to conventional heat processes, although there are notable exceptions. Spores of *C. botulinum* for example, have been found to be more resistant to gamma rays than are spores of a flat sour bacterium (No 1518) and a thermophilic anaerobe (TA. No 3814) (Frazier & Westhoff, 1988).

2.4.2 *Effect of food irradiation on sensory quality*

A limiting factor with regard to irradiation processing is the possible development of objectionable changes in the properties of some foods such as flavour, odour, colour, texture, and perceived freshness. However, using the treatment in association with other processes can counteract the negative effects of irradiation. Lower doses of irradiation accompanied by a refrigeration process are proving to be technically and economically feasible (Swart, Blignaut & Jooste, 1993).

Changes of the external appearance of food due to irradiation is attributed to the inability of cells injured by irradiation to repair the surface damaged at the time it is caused (Murray, 1990). However, it must be recognised that undesirable changes in foods by irradiation are no greater, and often less than when food is treated by other widely used methods of preservation, such as canning (Schubert, 1974).

The principal limiting factor in irradiation of vegetables is the loss of texture. Texture is affected in three possible ways: by weakening the rigid structural tissue; by altering the cell walls, hence reducing turgor; and by affecting processes of endogenous enzymes, either by releasing the enzymes from their normal locations into the plant tissue where they can attack carbohydrates or by altering the carbohydrate substrates so as to make them more susceptible to enzyme action (Urbain, 1986).

Detrimental changes in vegetables are apparent at low dosage levels, resulting not only in texture loss and partial pectin degradation but also in colour change/discolouration and loss of natural flavour (Ley, 1983). Unwanted volatile off-flavours and odours are produced on irradiating some of the food products. Some of these volatiles are formed from proteins and others from fats. The presence of these off-flavours and odours generally increase with increasing radiation dose (Schubert, 1974). These affect the acceptability of the meal and considerably could be the limiting to the

potential use of irradiation for ready meals containing vegetables such as cauliflower and mashed potatoes (Patterson *et al.*, 1998).

It has been shown that starch is highly susceptible towards degradation by irradiation. The major effects of irradiation on the carbohydrates found in foods are the same as those caused by cooking and other types of processing treatments (Murano, 1995b). According to Erasmus (1996) results, it was found that there was a decrease in the viscosities of the starch suspension and starch sauce. These results indicated that the starch used was highly susceptible in terms of viscosity reduction during irradiation. The pasting of the starch after irradiation; observed microscopically, indicated that changes in the viscosity of the starch product after irradiation are similar to the changes that occur when starch is being overcooked. In fact, irradiation cause breakdown of large polysaccharide chains, degradation of starch and cellulose into simple sugars, and formation of sugar acids, ketones, and other sugars from polysaccharides. The depolymerisation that occurs after irradiation is dependent on the irradiation dose applied (Satin, 1993). This could be the possible reason for the taste and textural changes that occurs in starchy foods.

2.5 Nitrite

Nitrite is an antimicrobial, conferring considerable bacteriological stability to a product, its activity increasing with lower pH. It also contributes to the cured food flavour, which differs from the taste of salty foods. Redox potential (Eh) also affects the activity of nitrite against some bacteria, for example, anaerobic conditions (reduced Eh) increase its inhibitory effect (Roberts, Woods, Payne & Cammack, 1991).

The question of relative importance of input versus residual nitrite in botulinal inhibition is basic to understanding the role of nitrite and determining the effect of various factors on nitrite inhibition. The input level of nitrite is important to be taken into consideration, and this is due to the

fact that (1) nitrite acts on the botulinal spores to cause inhibition (2) the input level of nitrite is responsible for curing the product and providing sufficient residual nitrite for effective botulinal inhibition (Roberts *et al.*, 1991).

The pH of a product is an important factor in determining both the effectiveness of nitrite and the fate of *C. botulinum*. A pH of 4.6 or lower is considered to be inhibitory to botulinal growth regardless of other factors. If the initial pH is below the inhibitory level, there is no problem. However, a pH which is above the inhibitory level for botulinal growth, may actually increase the potential botulinal risk in a temperature abused product. The rate of nitrite depletion is increased as pH is decreased. The rate of botulinal spore germination, on the other hand, might be expected to decrease as pH approaches an inhibitory level. Thus, more viable organisms would be present at a time when nitrite had been depleted below an inhibitory level (Christiansen, 1980).

Since heating destroys vegetative cells and many cured food products are subjected to a heating process of some degree, the organisms that are of most interest are those which are able to produce heat-resistant forms, i.e. spores. In particular, the potential for serious outbreaks of food poisoning by *C. botulinum* has resulted in a great deal of effort to determine the response of this organism and its spores to nitrite in both foods and laboratory media (Lück & Jager, 1995).

2.5.1 Mode of Action

Several scientists have demonstrated that nitrite has strong antimicrobial properties. They postulated the anticlostridial mechanism to consist of partial conversion of nitrite to hydroxylamine, which inactivates catalase and allows the accumulation of hydrogen peroxide, which destroys anaerobes (Tompkin, 1983; Jay, 1992).

Pivnick, Johnston, Thacker & Loynes (1970) investigated four possible roles of nitrite in shelf stable cured products: (1) Nitrite induces germination during the heating process and the germinated spores are killed by heat; (2) nitrite potentiates direct killing by heat without the need for prior germination; (3) nitrite increases the germination rates of spores that survive heating, and (4) these rapidly germinating spores are then inhibited by the nitrite remaining after processing and subsequently die before the nitrite has decreased appreciably. Of the four possible roles, nitrite at the level used commercially (200 µg/g) did not enhance destruction during heating or germination of spores during subsequent storage. Hence, the chief value of nitrite appears to be its ability to prevent growth from spores which survive and germinate following processing (Tompkin, 1983).

According to Woods, Wood & Gibbs, (1981), the pH dependence of nitrite was described by Grindley in 1929, who suggested that it could be due to nitrous acid being the active form of the preservative. Later, Tarr (According to Woods *et al.*, 1981) showed that the preservative action of nitrite in fish was greatly increased by acidification; in bacteriological medium inhibitory action of nitrite on several species of bacteria was shown to increase with decreasing pH, particularly at pH 6.0 and below. These results suggested that the undissociated nitrous acid was the active inhibitor (Woods *et al.*, 1981).

The evidence that nitrite can prevent the outgrowth of germinated spores suggests that nitrite inhibits one or more of the metabolic process that is required for growth. An important process that occurs in the cell is the oxidation of substrate with concomitant production of adenosine triphosphate (ATP), which can then be used subsequently as an energy source for the synthesis of new cellular material and hence growth (Jay, 1992).

The effect of nitrite on the metabolism, particularly the glucose metabolism, of cells of clostridia has been studied by Woods & Wood (1982). In these

organisms, an important source of ATP is the oxidation of pyruvate to acetate by the phosphoroclastic system.

According to Jay (1992), it appears that nitrite inhibits *C. botulinum* by interfering with iron-sulfur enzymes such as ferredoxin and thus preventing the synthesis of adenosine triphosphate (ATP) from pyruvate. It has been shown that sodium nitrite inhibits the phosphoroclastic systems of clostridia by interaction of nitric oxide formed from nitrite (Woods & Wood, 1982), and that the same occurs in *C. botulinum* whereby the effect results in the accumulation of pyruvic acid in the medium (Woods, Wood & Gibbs, 1981). The clostridia contain both ferredoxin and hydrogenase, which function in electron transport in the anaerobic breakdown of pyruvate to yield ATP, H₂ and CO₂. The ferredoxin in clostridia has a molecular weight of 6000 and contains eight iron atoms/mole and eight labile sulfide atoms/mole (Jay, 1992). The resistance of some microorganisms to nitrite inhibition is due to the fact that these organisms lack ferredoxin.

The phosphoroclastic reaction involves the breakdown of pyruvate with inorganic phosphate and co-enzyme A to yield acetyl phosphate. In the presence of adenosine diphosphate (ADP), ATP is synthesized from acetyl phosphate with acetate as the other product. In the breakdown of pyruvate, electrons are transferred first to ferredoxin and from ferredoxin to H⁺ to form H₂ in a reaction catalysed by hydrogenase. Ferredoxin and hydrogenase are both iron-sulfur enzymes (Jay, 1992).

Reddy, Lancaster & Cornforth (1983), subjected extracts of nitrite-ascorbate treated *C. botulinum* to electron spin resonance (ESR) and found that vegetative cells of *C. botulinum* contains iron-sulfur proteins that react with added nitrite to form iron-nitric oxide complexes, which in turn results in destruction of the iron-sulfur cluster. Inactivation of iron-sulfur enzymes (especially ferredoxin) by binding nitric oxide would certainly inhibit growth and is probably the mechanism of botulinal inhibition by nitrite in foods.

2.6 Preservation by combination processing

Hurdle technology (also referred to as combination processing, combination preservation, combination techniques or barrier technology) was developed as a new concept for the realisation of safe, stable, nutritious, tasty, and economical foods. It employs the intelligent use of a combination of different preservation factors or techniques to achieve multi-target, mild but reliable preservation effects. Many promising hurdles have been identified so far, but application of the concept in the food industry has been largely restricted to the meat sector. However, the concept was introduced to mild processing of fruits and vegetables (Grijspaardt-vink, 1994).

Hurdle technology deliberately combines existing and new preservation techniques to establish a series of preservative factors (hurdles) that the microorganisms in question are unable to overcome (“jump over”) (Leistner & Gorris, 1995). Different hurdles such as salt, acid and preservative effects combine to allow, for instance, a reduced thermal process or an increased storage time (Anderson, 1991). These hurdles include:

- Physical hurdles i.e. high temperature during processing, low temperature during storage, irradiation, Modified Atmosphere Packaging (MAP), aseptic packaging and packaging film;
- Physico-chemical hurdles i.e. water activity, pH, redox potential, preservatives;
- Microbial derived hurdles; i.e. competitive microflora, protective culture, bacteriocins, chitosan;
- Miscellaneous hurdles i.e. chlorine, chitosan.

These hurdles are applied at optimal range to achieve the preservation effect (Chirife & Favetto, 1992; Leistner & Gorris, 1995).

The concept of hurdle technology is by no means a novel process (Leistner, 1994) and is more or less unconsciously used in many traditional foods produced in developing countries with mild heat or without any application of heat (Leistner, 1994). The use of the hurdle technique is

very old and can be traced far back to ancient Egypt where the hurdle concept was used for mummy preservation, a process which comprised at least three different hurdles namely reduced water activity (a_w), increased pH, and preservatives (Chirife, Favetto, Ballesters & Kitic, 1991).

2.6.1 Principles of combination processing

The principle of hurdle technology is based on a crucial phenomenon known as the homeostasis of microorganisms. Homeostasis is the constant tendency of microorganisms to maintain the stability and balance of their internal environment. For food preserved by hurdle technology, the preservation is achieved by disturbing one or more of the homeostatic mechanisms of the microorganism, thereby preventing microorganisms from multiplying and causing them to remain inactive or even die (Grijspaardt-vink, 1994).

It has been suspected that different hurdles in a food could have either an additive effect on the stability or may act synergistically to such an extent that combinations of different preservative factors will deliberately disturb several of the homeostasis mechanisms simultaneously (Grijspaardt-vink, 1994). The synergistic effect becomes possible if different hurdles applied in food hit at the same time different targets (e.g. cell membrane, DNA, enzymes, pH, a_w and Eh) within the microbial cell, and thus disturb the homeostasis of the microorganisms present in several respects. This multi-targeted approach is the essence of hurdle technology, because if small hurdles with different targets are selected, a minimal but most effective preservation of foods could be achieved (Grijspaardt-vink, 1994; Leistner & Gorris, 1995).

Studies have also shown that mild and effective preservation based on an intelligent selection of different hurdles, turned out to be more effective than the application of one hurdle, because it allows the use of hurdles of lower intensity, which have the least effect on product quality.

2.6.2 Significance of combination processing

Hurdle technology is not only a safety tool but also encompasses quality aspects of foods (Leistner, 1994). These aspects are noted in cases where foods preserved by combined methods remain stable and safe even without refrigeration, and are high in sensory and nutritive properties due to the gentle processes applied (Alzamora, Tapia, Argaiiz & Well, 1993). Foods preserved by one preservation technique tends to have many limitations especially in terms of their sensory qualities and somehow the microbiological qualities of the particular food (Aguilera & Chirife, 1994).

The spoilage and poisoning of foods by microorganisms is a problem that is increasing worldwide despite the wide range of preservation techniques employed. In fact, the current consumer demand for food that appears more natural and fresh thus prompting food manufacturers to more mild preservation techniques, could be stimulating this trend. Thus for the benefit of these food manufacturers there is a need for new or improved methods that allow for the production of stable and safe food (Leistner, 1995).

Hurdle technology is also widely used in food design for making new products according to specific needs. For instance, if energy preservation is the goal, then energy consuming hurdles such as refrigeration can be replaced by hurdles such as a_w , pH or redox potential (Eh), which do not demand energy and still ensure a stable and safe food (Leistner, 1978).

2.6.3 Irradiation combined with other preservation techniques

When any irradiation process is used that does not result in complete sterilisation, serious consideration must be given to decide which pathogenic organisms might develop in the food. Post-irradiation conditions must be such as to inhibit them (e.g. by curing, drying or storage at sufficiently low temperature) (Desrosier, 1977).

Treating some foods with ionising radiation alone may not produce the desired results. Reasons for such situations are (1) the dose of ionising energy required to produce a specific desired effect may produce other unacceptable changes in the food, (2) ionising energy alone does not produce the desired effect (3) applying the dose required to produce the desired effect is too expensive. In such situations, a combination of processes such as irradiation is used along with some other treatment may produce the desired results. A number of potentially useful combinations of processes have been developed (Gould, 1989).

Treatments with ionising radiation in combination with refrigeration are especially valuable. Ionising radiation at substerilising doses adds to the preservative effect of refrigeration on fresh foods, and the combination of processes is better than either process used alone. Ionising radiation would not be effective alone because more than microbiological spoilage (for example, autolysis and oxidation) is involved in the deterioration of these foods. Obilana (1998) found that it was possible to produce a RTE spinach based relish and sorghum porridge meals with a shelf life of at least 7 d by treating the product with 10 kGy irradiation dose and storing the meal at 5 °C. In particular perishable foods, doses of the order of hundreds of kilorads can decrease the rate of microbial spoilage several-fold. However, the extension of storage life of such foods is appreciable only if the irradiated foods are stored under conditions commonly used in commercial storage (Gould, 1989).

According to Desrosier (1977), the temperature of storage should be low enough to prevent the growth in irradiated foods of all pathogens, especially those with spores which survive irradiation. *C. botulinum* type E, for example, will grow at a temperature of 5 °C and perhaps lower.

Heat is the most widely used method of food preservation. It is effective in reducing or eliminating microbial contaminants, making products safe, and extending their shelf life. The only problem with heat is that it can have a significant effect on food quality. This effect is based on the ability of heat

to coagulate proteins, inactivate enzymes, and destroy nutrients, all of which can alter the texture, flavour and odour of a product. The use of thermo-radiation (heat + irradiation) can result in an effective combination treatment, compared with heat or irradiation alone (Murano, 1995a).

The studies by Minnaar (1993) and Minnaar, Taylor & McGill (1995) in low acid foods showed that heat-irradiation combination processing could be used to produce high quality shelf stable products and hence offer a feasible alternative to thermal processing in terms of quality. Generally, heat in combination with ionising radiation reduces the required dose of ionising energy in some instances. For example, the autolytic enzymes in meats are not inactivated completely even with doses of ionising energy of 200 kGy (a dose approximately five times greater than that needed to produce sterile products). Nevertheless, heating meats to 70 to 80 °C inactivate the enzymes and avoids the need for the very high doses of ionising radiation energy (Task Force Report, 1989). Therefore, mild heat treatment (70 to 110 °C core temperature) for example, for certain products is beneficial because it fosters the sensory and nutritional properties of the products (Leistner & Gorris, 1992).

Vacuum packaging is advantageous in preserving some foods because atmospheric oxygen is required for the growth of some spoilage organisms. Moreover, oxygen interacts chemically with some constituents in foods, notably fats, causing oxidation and off-flavours. Treatment of certain foods with ionising radiation is of value in reducing the numbers of spoilage microorganisms, but when done in the presence of atmospheric oxygen, it increases the reactivity of the oxygen and hastens the development of off-flavours in foods that are prone to this problem. Vacuum packaging avoids the oxygen problem and reduces the dose of ionising energy required (Task Force Report, 1989).

Modified Atmosphere Packaging (MAP) of foods is one of the new food preservation technologies. The quality of such foods is superior to that of thermally processed products, and their shelf stability has allowed countries

such as the United States to better compete in foreign markets. The combined effect of irradiation and this packaging method can result in production of products of higher quality than those packaged in an atmosphere where oxygen predominates. However, survival of microorganisms in such products is not as predictable as one may imagine (Murano, 1995a).

Obilana (1998) found that it was possible to produce a safe sorghum porridge and spinach RTE meal with a shelf-life of at least 7 days at 5 °C using a combination of MAP (84.5% N₂ + 15.5% CO₂) with irradiation at a target dose level of 10 kGy provided there is low initial microbial load on the raw materials and finished product, no cross-contamination during processing and by ensuring the maintenance of the cold chain throughout processing, storage, distribution and final end-use.

Reducing the water content of foods (more specifically the water activity) is another way of inhibiting the multiplication of microorganisms. High concentrations of sugar or salt, as in fruit jellies or salted fish, reduce the a_w and have a preservative effect similar to drying. In some instances, ionising energy can be used in combination with decreased water content or reduced a_w to preserve food with benefits in terms of both quality and economics. The preservation of partially dried shrimp with ionising energy is an example (Gould, 1989).

2.6.3.1 Irradiation combined with nitrite

Certain chemicals can also be used to advantage in combination with ionising energy. Salts are effective in reducing the water activity inhibit microbial growth. Nitrite has been mentioned for its effect in preventing spores of *C. botulinum* from developing into actively multiplying vegetative cells that produce the botulinum toxin (Task Force Report, 1989).

Sodium nitrite has been used in producing cured products in combination with salt and other substances in bacon, ham, and certain meat products in

processes that impart a characteristic colour and flavour, reduce oxidative changes, and retard the growth of microorganisms, including *C. botulinum*. Under refrigeration storage, cured products can have a shelf life as long as 50 d. In the other hand, irradiation has a potential of destroying life forms with a relatively low energy and therefore results to a relative small amount of chemical change (Deeble *et al.*, 1990). The potential application of ionising irradiation in combination with sodium nitrite to products is for maintaining the shelf life, while allowing a reduction in the quantity of sodium nitrite used.

The desire to reduce the amount of sodium nitrite used has resulted from the fact that nitrosamines are formed when nitrite interacts under appropriate conditions with certain nitrogenous compounds present in flesh foods and formed in them during digestion and during frying at high temperatures. Some nitrosamines have been found to be potential animal carcinogens. Research has shown that treatment with ionising irradiation allows a substantial reduction in the amount of sodium nitrite needed in curing without loss of the traditional flavour and colour (Task Force Report, 1989). Higher level of sodium nitrite can be applied as long as it does not exceed the legal residual level; 200 mg.kg⁻¹ residual nitrite is permitted in meat products in South Africa. Another rational for the combination is that when high irradiation doses cannot be applied to inactivate spores due to off-flavours development and loss of colour and texture; low irradiation doses can be used in combination with other preservation techniques.

CHAPTER 3

OBJECTIVES

3.1 Primary objective

The primary objective of this research was to determine the effect of a combination process (sodium nitrite and irradiation) on the safety of a ready-to-eat (RTE) spinach (morôgo) and sorghum porridge meal.

3.2 Secondary objectives

The secondary objectives of this research were:

1. To conduct consumer acceptability tests to establish the maximum acceptable irradiation dose for spinach (morôgo) and sorghum porridge RTE meal.
2. To determine the effect of washing (in chlorinated water), blanching (in two water changes) of spinach, and cooking (mild heat treatment) on the survival of inoculated *Clostridium sporogenes* spores in spinach and sorghum porridge.
3. To determine the effect of sodium nitrite on the survival of inoculated *Clostridium sporogenes* spores in the spinach (morôgo) and sorghum porridge meal components stored at 10 °C for 12 days.
4. To determine the residual sodium nitrite levels in spinach (morôgo) and sorghum porridge meal components after cooking and irradiation processes.
5. To determine the effect of sodium nitrite (at different levels) in combination with a given dose of irradiation on the survival of the inoculated *Clostridium sporogenes* spores in spinach and sorghum porridge meal components stored at 10 °C for 12 days.

CHAPTER 4

MATERIALS AND METHODS

4.1 Experimental design

To determine the optimum combination processing techniques of RTE sorghum porridge and spinach (morôgo) meal, the research was sub-divided into preliminary experiments and the main experiment.

4.1.1 Preliminary experiments

4.1.1.1 Sensory acceptability test

To investigate the effect of different irradiation doses (0, 10, 20 and 30 kGy) on the sensory acceptability of the meal, a consumer acceptance test was performed for irradiated spinach relish and sorghum porridge samples. The objective of conducting the consumer acceptability test was to establish the highest irradiation dose, which was still organoleptically acceptable for producing a RTE meal.

Spinach (morôgo) and sorghum porridge RTE meal samples were prepared at the Department of Food Science, University of Pretoria and taken to ISO-STER, Isando, Kempton Park for irradiation one day before the sensory evaluation. The following procedures were followed:

- *Sensory test method*

A hedonic rating scale was used to determine the consumer acceptability of the RTE meal. An evaluation form (Appendix 2) with a 9 point rating scale

ranging from "dislike extremely" to "like extremely" was used by participants to assess the acceptability of the sensory quality characteristics (appearance, texture and taste), and the overall acceptability of the RTE meal irradiated at different doses. Except for overall acceptability, meal components were assessed for appearance, texture and taste separately.

- *Test panel*

The consumer test panel comprised 50 students and personnel from the University of Pretoria. Recruitment was carefully done, only individuals that were familiar with, and regularly consumed this type of meal, were chosen.

- *Test location*

The sensory evaluation test was performed in the Home Economics Dining room, at the Department of Home Economics (University of Pretoria). At least 25 participants were accommodated at a time, and they were divided into two groups of which each group had a session to attend. The conditions in the testing room were controlled and participants were supervised during the test session. Individual assessment was followed throughout the test i.e. communication between panellists was not allowed. Daylight conditions were used throughout the testing period.

- *Test procedure*

Samples (approximately 30 g sorghum porridge and 25 g spinach relish) were portioned into ramekins (porcelain containers) and covered with aluminium foil. Samples were then reheated in an AEG oven to an internal temperature of 55 - 60 °C before being served. Before serving, samples were coded with 3 digit numbers and served in a randomised fashion to minimise bias. Panellists were familiarised with the evaluation forms and left to evaluate

samples following the form's description (Appendix 2). Explanation of the terms used to describe various characteristics of samples was given to panellists where it was necessary.

4.1.1.2 Effect of blanching, washing and cooking on the *Clostridium sporogenes* spores

The objectives of these preliminary experiments were to determine the effect of washing in 250 mg.l⁻¹ of NaOCl [prepared by diluting ACE bleach (3.5% m/v) produced by Procter and Gamble SA, Kempton Park, South Africa], blanching (in two water changes at 77 °C for 6 min) and cooking (mild heat treatment for 25-30 min) on the survival of the inoculated *Clostridium sporogenes* spores in spinach relish and sorghum porridge.

- *Experiment 1*

Experiment 1 investigated the effect of washing with chlorinated water on the survival of the inoculated *Clostridium sporogenes* spores in spinach morôgo leaves. *Clostridium sporogenes* spores (1 ml of a suspension containing 10⁷ spores/ml) were inoculated into 100 ml water (using Eppendorf multipipette 4780). A shredded spinach sample (100 g) was then immersed into the inoculated water and mixed for 10 min to allow a uniform distribution of spores to the sample. The raw inoculated spinach leaves were then washed in chlorinated water (with NaOCl concentration of 250 mg.l⁻¹ available Cl₂). The number of surviving *Clostridium sporogenes* was then determined.

- *Experiment 2*

Experiment 2 investigated the effect of blanching on the survival of *Clostridium sporogenes* spores in spinach relish. The inoculated and washed raw spinach (morôgo) leaves (250 mg.l⁻¹) were blanched in two water changes at 77 °C for 6 min. The number of surviving *Clostridium sporogenes* was then determined.

- *Experiment 3*

The effect of cooking on the survival of the inoculated *Clostridium sporogenes* spores in spinach morôgo leaves and sorghum porridge RTE meal components was carried out in Experiment 3. The raw inoculated, washed and blanched spinach (morôgo) leaves were cooked for approximately 25 to 30 min. The number of surviving *Clostridium sporogenes* spores was determined after cooling the samples to room temperature.

For sorghum porridge, 100 ml of water was inoculated with 10^7 spores/ml. The water was then brought to boil to allow a uniform distribution of spores in the sample. The porridge was cooked for 25 to 30 min following the method as described by Duodu *et al.* (1999). Samples were then cooled to room temperature and the spore enumeration was followed thereafter.

4.1.2 *Main experiment*

The objective of the main experiment was to determine the effect of a combination process of sodium nitrite at different levels (0, 50, 100, 150, and 200 mg.kg⁻¹) and the established irradiation dose on the survival of the inoculated *C. sporogenes* spores and the subsequent growth of the vegetative cells in the RTE spinach relish and sorghum porridge meal. The analyses (clostridia counts) were carried out over a period of 12 d, i.e. day 1, 6 and 12. Samples were stored at 10 °C during the period of analysis in order to determine the safety of the ready-to-eat meal if the meal was temperature abused. The residual sodium nitrite was also determined in this phase by investigating the effect of cooking and the effect of cooking and irradiation on the residual nitrite level. Two replicates were prepared in each experiment and each experiment was performed in triplicate.

4.1.2.1 Effect of nitrite alone on the survival of inoculated *C. sporogenes* spores

Sorghum porridge and spinach morôgo samples were prepared following the method described above (see section 4.2). In this experiment, samples were not irradiated. The analyses to determine the survival of the inoculated *Clostridium sporogenes* spores were then carried out over a period of 12 d. Samples were stored at 10 °C during the period of analysis.

4.1.2.2 Effect of cooking, and cooking and irradiation on the residual nitrite level in a RTE meal

Sorghum porridge and spinach morôgo samples were prepared following the method described above (see section 4.2). The AOAC Official Method of Analysis (1995) was then followed to determine the effect of cooking, and the effect of cooking and irradiation of spinach based relish and sorghum porridge on the residual nitrite level.

4.1.2.3 Effect of nitrite in combination with irradiation on the survival of the inoculated *C. sporogenes* spore in a RTE meal

Sorghum porridge and spinach (morôgo) samples were prepared following the method described above (see section 4.2). Clostridia counts were then carried out to determine the survival of the inoculated *Clostridium sporogenes* over a period of 12 d. Samples were stored at 10 °C during the period of analysis.

4.2 Materials

Sorghum flour (Super Mabela sorghum flour) was supplied by Nola (Pty) Ltd., Randfontein. Spinach (morôgo) leaves were obtained from a producer of spinach in the Pretoria area. Tomato onion mix (FARMGIRL Maxim Packers,

South Africa), white pepper (Pick 'n Pay's Choice) and salt were purchased from the Pick 'n Pay Supermarket, Hatfield, Pretoria. Polystyrene trays were obtained from Pick 'n Spice, Pretoria. Polyethylene bags (polyvinylchloride-coated polyester; 15 μm barrier abuse layer laminated with 50 μm linear low density polyethylene) were obtained from Cryovac (Pty) Ltd, Kempton Park.

4.3 Preparation of a RTE sorghum porridge and spinach relish

Sorghum porridge and spinach based relish meals were prepared following the method adapted from Duodu, Minnaar & Taylor (1999). Figure 1 gives a summary of the process description. Storage temperature of 10 $^{\circ}\text{C}$ was chosen in order to determine the safety of the RTE food in case of temperature abuse during storage.

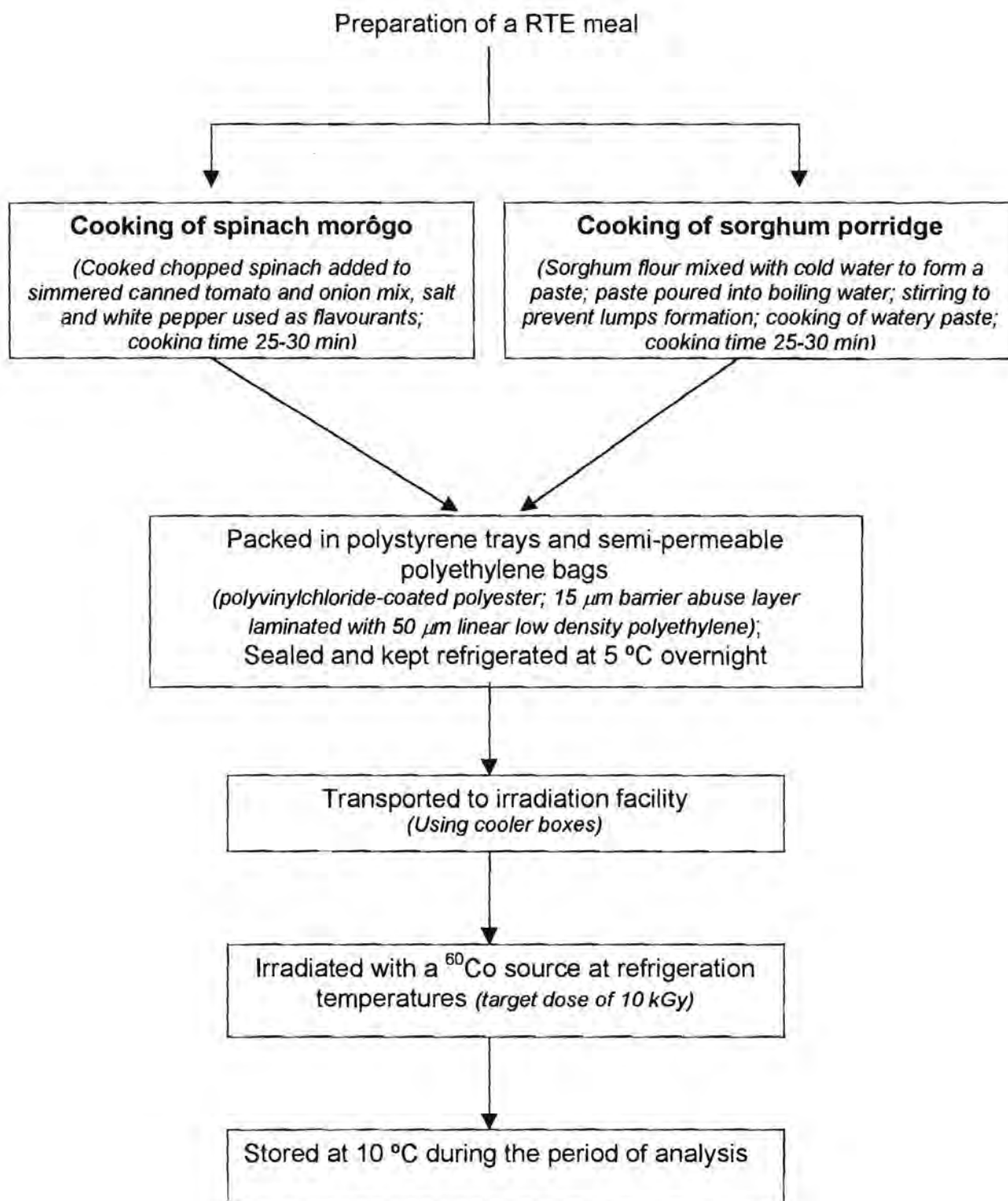


Figure 1 Flow diagram of preparation and processing of the RTE spinach morôgo and thick sorghum porridge meal

4.3.1 Samples for inoculated pack studies

4.3.1.1 Spinach relish

Young soft tender spinach (morôgo) leaves were rinsed with water to remove dirt and soil particles, and chopped up with a knife.

For preparation of the spinach relish, 50 ml of water was inoculated with 1 ml of a spore suspension containing 10^7 spores/g of *C. sporogenes* and sodium nitrite at different concentrations (0, 50, 100, 150, and 200 mg.kg⁻¹) was then added. The mixture was brought to a boil to allow the uniform distribution of spores and sodium nitrite into the water. Washed and chopped spinach leaves (100 g) were added to the boiling water, and then 0.6 g of salt was added. The mixture was cooked (stirring occasionally) until the water had totally evaporated (approximately 7-10 min). In a clean pan, 60 g of tomato onion mix (Farmgirl, Maxim Packers, South Africa) was simmered for 10 min. White pepper (0.6 g) was added for taste. The cooked spinach was then added and the mixture cooked for a further 25 to 30 min.

4.3.1.2 Sorghum porridge

For the sorghum porridge, 3 ml of a spore suspension containing 10^7 spores/g were inoculated into 300 ml of water followed by addition of sodium nitrite at different concentrations (0, 50, 100, 150, and 200 mg.kg⁻¹). The water was brought to a boil in a clean saucepan to allow a uniform distribution of spores and nitrite in the water. Sorghum meal (135 g) was mixed with 60 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 (boiling) temperature until a thick paste was formed (approximately 25 to 30 min).

4.3.1.3 Sample irradiation

The sorghum porridge and the spinach relish were left (approximately 1.5 h) to cool to ambient temperature (20 to 25 °C), then dished out into polystyrene trays. The meal was packed in semi permeable polyethylene bags and sealed. The packed meal samples were stored overnight at 5 °C, and transported in cooler boxes to ISO-STER irradiation facility at Isando (Kempton Park, RSA), where they were exposed to a ⁶⁰Co source at refrigeration temperature. Samples were irradiated to a target dose of 10 kGy (the dose decided on after the sensory analysis test). However, samples received irradiation doses of 13.8, 10.4, 10.0 and 12.3 kGy for the first to the fourth replicates respectively (main experiment) at a dose rate of 1.4 kGy/h. Samples of the product were taken immediately after irradiation and the microbial analyses were performed on day 1, 6 and 12 after irradiation.

4.3.2 Samples for sensory evaluation

The same procedure was followed to prepare samples for the sensory evaluation test. The only exception was that the samples were not inoculated with spores, were not treated with nitrite and were irradiated at 0, 10, 20 and 30 kGy.

4.4 Preparation of spore suspensions for inoculated pack studies

Spores of *Clostridium sporogenes* locally isolated from irradiated meat products (isolates Cl₃, Cl₅ and Cl₁₀) were produced by the biphasic method of Anellis, Berkowitz, Kemper & Rowley (1972). The spores were propagated in broth composed of 5% (m/v) Tryptone (Oxoid), 0.5% Peptone (Biolab), and 0.125% K₂HPO₄ (Merck), and adjusted to pH 7.5 with 5 M KOH before autoclaving. Filter-sterilised NaHCO₃ 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 flasks, autoclaved, cooled rapidly to 20 to 30 °C, and overlaid aseptically with a sterile 2% $(\text{NH}_4)_2\text{SO}_4$ liquid phase 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 7.0) 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. The spores were 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 4.3

4.5 Microbiological analysis

4.5.1 Sampling and preparation of dilutions

The RTE meal samples were aseptically sampled by weighing 20 g well-mixed sample into a sterile stomacher bag. To each bag 180 ml of sterile 0.1% peptone water (diluent) were added and then placed in the stomacher (supplied by Art Medical Equipment (Pty) Ltd, Johannesburg, South Africa) and the contents macerated for 0.5 to 1 min. Dilutions (10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6}) were made by transferring 1 ml of successive serial dilutions into McCartney bottles containing 9 ml of sterile peptone water. All dilutions were vigorously shaken on a vortex machine in order to obtain a homogeneous mixture for plating.

4.5.2 Enumeration of *C. sporogenes*

Clostridium sporogenes were enumerated using a modified version of the methods described by Anellis, Shattuck, Rowley, Ross, Whaley & Dowell (1975). Cells were not heat shocked because the preparation of samples involved a cooking process whereby the temperature and time used are enough to activate spore germination. Peptone P (Biolab) was used instead of Thiotone (BBL). The following culture media and reagents were used:

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

Enumeration:

One-millilitre aliquots of the various dilutions of the samples were inoculated into each of the triplicate tubes (11 by 202 mm) containing 0.25 ml of filter-sterilised 0.75% NaHCO₃, and sterilised molten agar medium at 45 °C was then added to the tubes. Pouring of the agar into the tubes produced uniform mixing of the spores by the resulting vortex action with the tube contents. The contents of the tubes were then sealed with 1.5 to 2 cm of the same medium, followed by incubation at 35 ± 2 °C for 48 h. The numbers of colonies formed in the triplicate tubes were counted and the counts averaged. Each sample was enumerated in duplicate.

4.6 Determination of residual sodium nitrite

Residual sodium nitrite levels in RTE sorghum porridge and spinach relish were determined using the AOAC Official Method of Analysis (1995).

4.6.1 Reagents and apparatus

- (a) NED reagent: Exactly 0.2 g N- (1-naphthyl) ethylenediamine.2HCl was dissolved in 150 ml 15% (volume/volume) CH₃COOH. The mixture was filtered (Whatman No.4) and stored in a glass-stoppered brown glass bottle.
- (b) Sulfanilamide reagent: Exactly 0.5 g sulfanilamide was dissolved in 150 ml 15% CH₃COOH. The mixture was filtered and stored in a glass-stoppered brown glass bottle.
- (c) Nitrite standard solutions: (1) *stock solution* - 1000 mg.l⁻¹ NaNO₂ i.e. 1 g NaNO₂ dissolved in distilled water and diluted to 1 l. (2) *Intermediate solution* - 100 mg.l⁻¹ NaNO₂ i.e. 100 ml stock solution diluted to 1 l with distilled water. (3) *Working solution* - 1 mg.l⁻¹ NaNO₂ i.e. 10 ml intermediate solution was diluted to 1 l with distilled water.

- (d) Filter paper (Whatman No.4): The test for nitrite contamination was done by analysing 3-4 papers, at random, throughout the box. Approximately 40 ml of distilled water was filtered through each paper. Exactly, 4 ml of sulfanilamide reagent was added, mixed, and left to stand for 5 min, then 4 ml NED reagent added, mixed and left for 15 min. If any of the papers tested positive, the entire box was discarded.

4.6.2 Determination of sodium nitrite

Exactly 5 g finely comminuted using a blender (supplied by Waring Commercial Blender, New Hartford, USA) and thoroughly mixed sample was weighed into a 50 ml beaker. Approximately 40 ml of distilled water heated to 80 °C was added and the mixture was thoroughly mixed with a glass rod, taking care to break up all lumps, and transferred to a 500 ml volumetric flask. The beaker and rod were washed thoroughly with successive portions of the hot water, adding all washings to the flask. Enough hot water was added to bring the volume to approximately 300 ml. The flasks were transferred to a steam bath and left to stand for 2 h while being shaken occasionally. The mixture was cooled to room temperature and diluted to volume with distilled water. The mixture was then filtered and 2.5 ml sulfanilamide reagent was added to an aliquot containing 5 - 50 µg NaNO₂ in a 50 ml volumetric flask, and mixed. After 5 min, 2.5 ml NED reagent was added, mixed and then diluted to volume. The mixture was left for 15 min to allow colour development. A portion of solution was transferred to a photometer cell (Milton Roy Spectronic 20D supplied by Milton Roy Company, USA) and the Absorbance at 540 nm was determined against a blank of 45 ml H₂O, 2.5 ml sulfanilamide reagent, and 2.5 ml of NED reagent.

Determination of nitrite present was done by comparison with a standard curve (Appendix 1) prepared by adding 10, 20, 30, and 40 ml working standard solution to a 50 ml volumetric flask then, 2.5 ml sulfanilamide reagent was

added and mixed. The process proceeded as described above, beginning "After 5 min, ..."

4.7 Statistical analysis

Statistical analysis on the sensory analysis test results was done using a Chi square test. For all other results, the analysis of variance (ANOVA) was carried out by using a Statistica Version 5.0 from the Microsoft Corporation and the least significant difference test (LSD-test) were used to determine whether a difference existed between means of treatments. All comparisons were done at a level of 5 % significance.

CHAPTER 5

RESULTS

5.1 Preliminary experiments

5.1.1 Sensory evaluation

Table 2 and Figures 2-8 show the effect of different irradiation doses on the sensory characteristics of the ready-to-eat sorghum porridge and spinach based relish meal. Table 2 shows the mean values of the appearance, texture, and taste of the spinach and sorghum porridge components, and the overall acceptability of the meal; the attributes that were used to describe the sensory characteristics of the two meal components subjected to four different irradiation doses (0, 10, 20 and 30 kGy). Figures 2-8 show the distribution of panellists for liking the four different irradiated samples. Comments of panellists are summarised in a table in Appendix 4.

In general, for the spinach relish component of the meal, there was no significant difference ($p < 0.05$) between the appearance, texture and taste of samples irradiated at 0, 10 and 20 kGy (Table 2). In all attributes the sample irradiated at 30 kGy was significantly less liked than the control sample. However, there were no significant differences ($p < 0.05$) in the appearance, texture and taste between spinach samples irradiated at 20 and 30 kGy. The distribution of panellists for liking the four samples irradiated at different irradiation doses showed that a lower percentage of panellists perceived the control sample as negative compared to other samples (Figures 3, 5 and 7). The distribution also showed that in general the percentage of negative response and the neutral response (dislike slightly to like slightly) increased with increased irradiation dose while the percentage of positive response (like moderately to like extremely) decreased with increased irradiation dose. The mean score values decreased with increased irradiation doses.

With the sorghum porridge component a different trend for the appearance, texture and taste was observed (Table 2 and Figures 2, 4 and 6). There was a significant difference ($p < 0.05$) in the appearance, texture and taste between the control sample and all other samples (Table 2). Significant differences ($p < 0.05$) also existed in the appearance and taste between samples irradiated at 10 kGy and other samples. However, there was no significant difference ($p < 0.05$) in the appearance and taste between samples irradiated at 20 and 30 kGy. There were no significant differences ($p < 0.05$) in the texture between samples irradiated at 10 and 20 kGy.

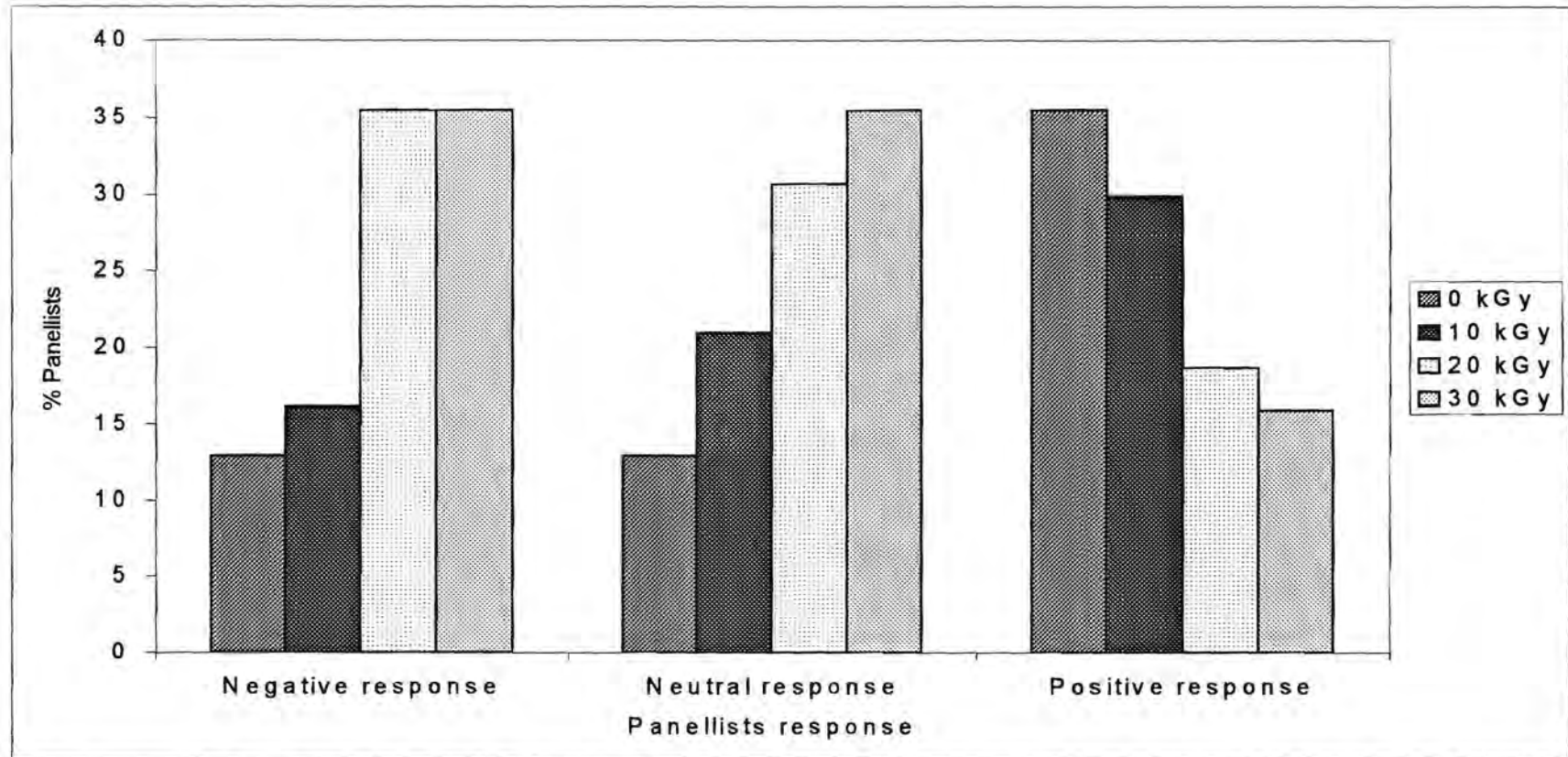
The distribution of panellists for liking the four samples irradiated at different irradiation doses showed a similar trend as that observed in spinach (Figures 2, 4 and 6). Percentages of negative and neutral responses increased as the irradiation doses increased, while the percentage of positive responses decreased with increased irradiation dose. The mean score values showed an inverse relationship with irradiation; the scores decreased as the irradiation doses increased.

The effects of different irradiation doses on the overall acceptability of the meal are given in Table 2 and Figure 8. There was a significant difference ($p < 0.05$) in the overall acceptability of the meal between the control sample and other samples. No significant differences ($p < 0.05$) existed in the overall acceptability between samples irradiated at 20 and 30 kGy, and the 10 kGy samples did not show a significant difference to the samples irradiated at 20 kGy. However, there was a significant difference in the overall acceptability between the samples irradiated at 10 kGy and samples irradiated at 0 and 30 kGy. The distribution of panellists for liking the four samples irradiated at different irradiation doses showed a similar trend to that obtained in the two components of the meal (Figure 8).

Table 2 Effect of different irradiation doses on the sensory characteristics³ of the RTE spinach relish and sorghum porridge meal

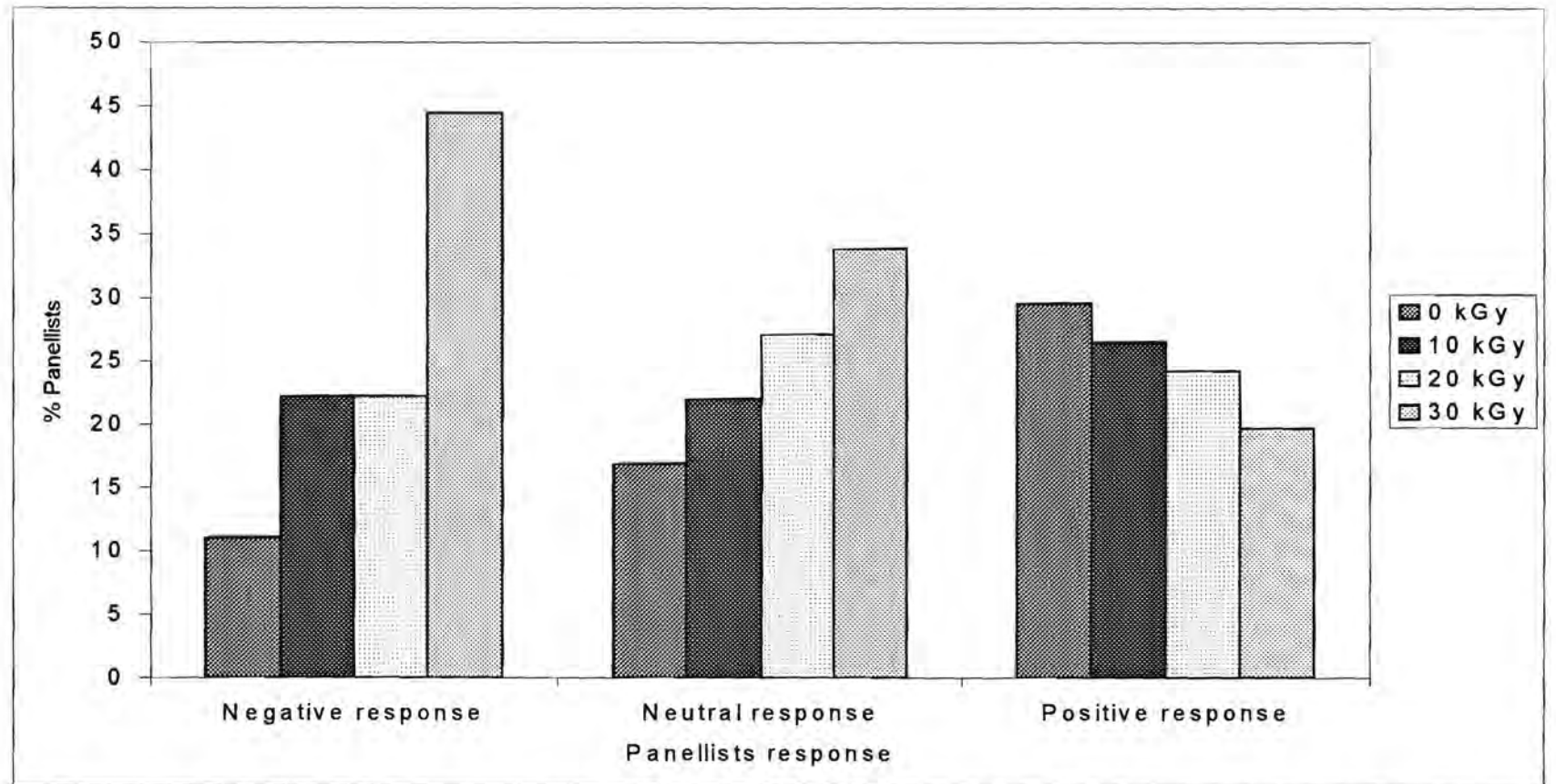
Treatments (kGy)	Appearance ¹ Porridge	Appearance ¹ Spinach	Texture ¹ Porridge	Texture ¹ Spinach	Taste ¹ Porridge	Taste ¹ Spinach	Overall ¹ acceptability of meal
0	7.26 ^c (±1.93) ²	7.20 ^{bc} (±1.62)	7.16 ^c (±1.99)	7.12 ^b (±1.59)	6.76 ^c (±2.38)	7.28 ^b (±1.70)	7.20 ^c (±1.35)
10	6.36 ^b (±1.95)	6.74 ^{ab} (±1.72)	6.16 ^b (±2.16)	7.12 ^b (±1.59)	5.70 ^b (±2.44)	7.08 ^b (±1.78)	6.43 ^b (±1.80)
20	5.50 ^a (±2.17)	6.58 ^{ab} (±1.68)	5.64 ^b (±2.24)	6.80 ^{ab} (±1.50)	4.68 ^a (±2.21)	6.56 ^{ab} (±1.94)	5.96 ^{ab} (±1.70)
30	5.30 ^a (±2.20)	6.30 ^a (±1.83)	4.64 ^a (±2.63)	6.30 ^a (±2.21)	3.82 ^a (±2.11)	6.10 ^a (±2.13)	5.41 ^a (±1.91)

- 1 Mean values in the same column with different letters differ significantly from each other ($p < 0.05$)
 2 Standard deviation is given in brackets
 3 Mean values of the sensory attributes: 1 = Dislike extremely; 9 = Like extremely



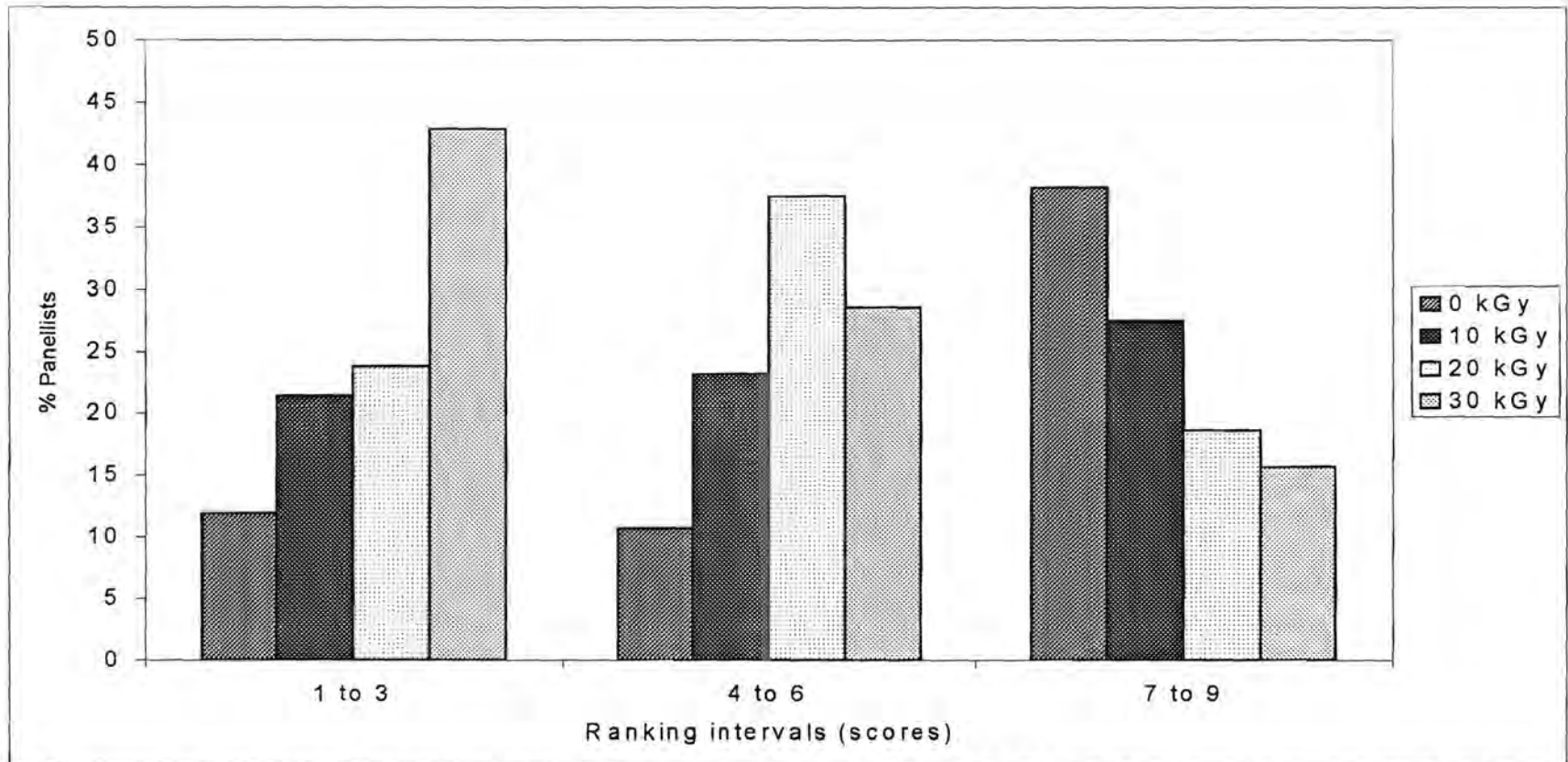
Negative response (Dislike extremely to dislike moderately); Neutral response (Dislike slightly to like slightly); Positive response (Like moderate to like extremely)

Figure 2 Percentage of positive, neutral and negative responses for the appearance of the sorghum porridge component of the RTE meal irradiated at different doses.



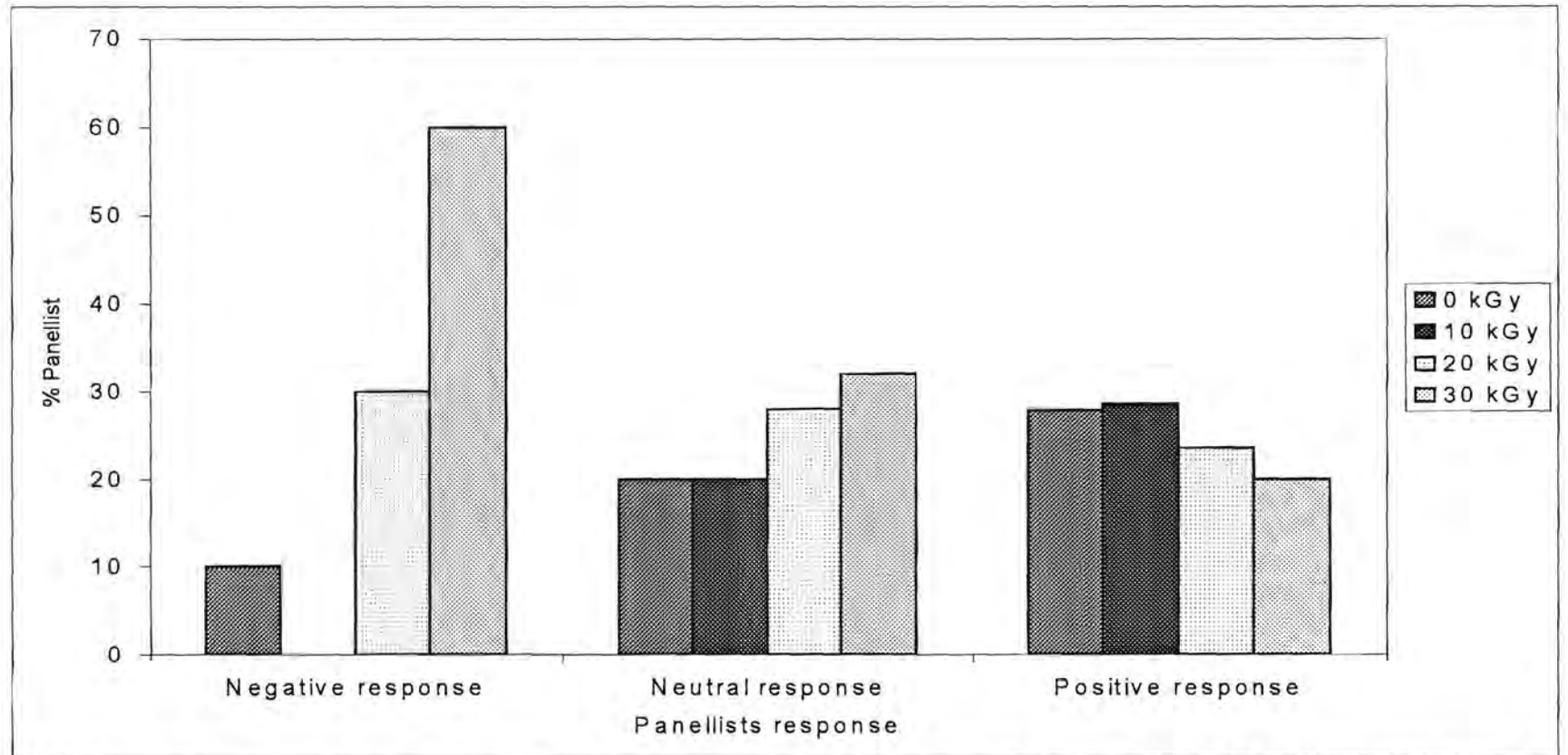
Negative response (Dislike extremely to dislike moderately); Neutral response (Dislike slightly to like slightly); Positive response (Like moderate to like extremely)

Figure 3 Percentage of positive, neutral and negative responses for appearance of the spinach component of the RTE meal irradiated at different doses



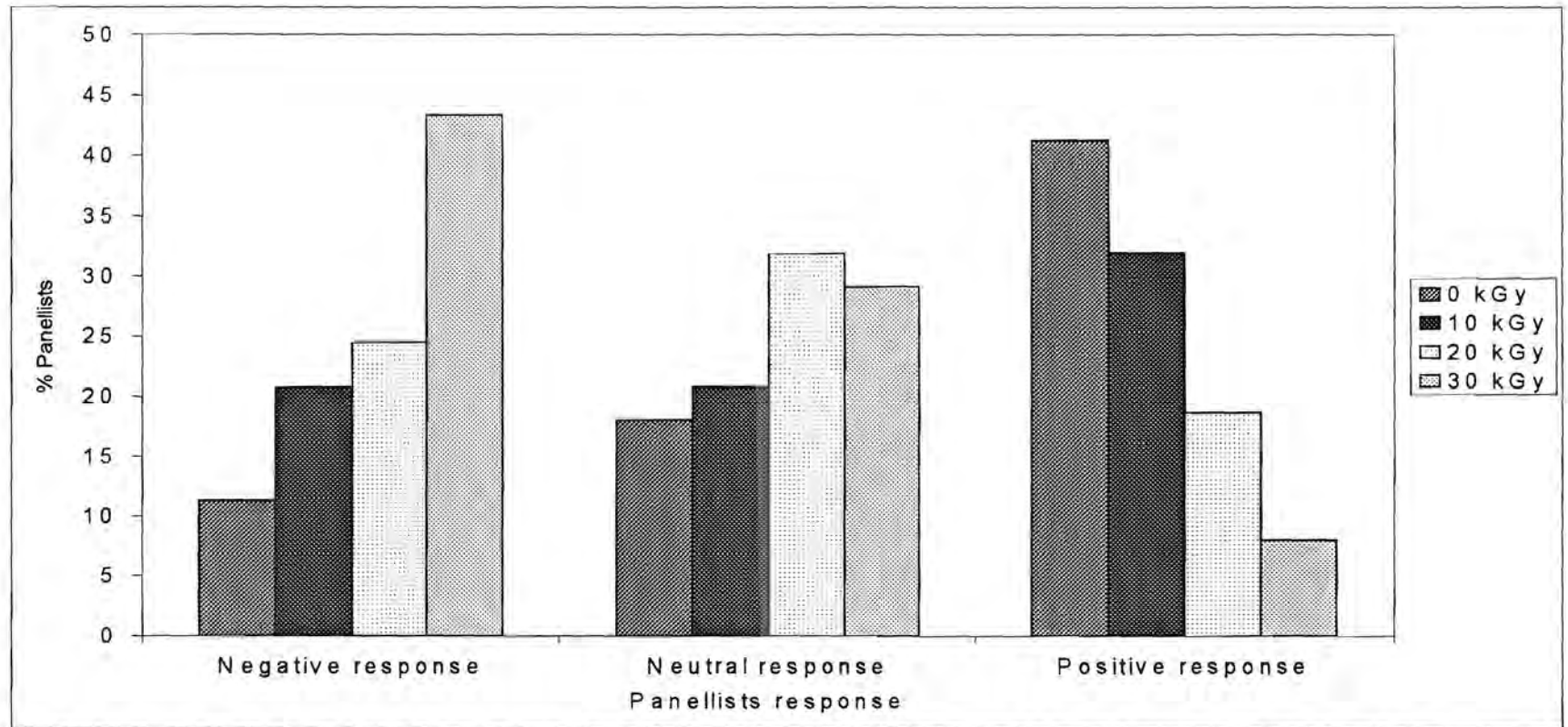
Negative response (Dislike extremely to dislike moderately); Neutral response (Dislike slightly to like slightly); Positive response (Like moderately to like extremely)

Figure 4 Percentage of positive, neutral and negative responses for the texture of the sorghum porridge component of the RTE meal irradiated at different doses



Negative response (Dislike extremely to dislike moderately); Neutral response (Dislike slightly to like slightly); Positive response (Like moderately to like extremely)

Figure 5 Percentage of positive, neutral and negative responses for the texture of the spinach relish of the RTE meal irradiated at different doses



Negative response (Dislike extremely to dislike moderately); Neutral response (Dislike slightly to like slightly); Positive response (Like moderately to like extremely)

Figure 6 Percentage of positive, neutral and negative responses for the taste of the sorghum porridge component of the RTE meal irradiated at different doses

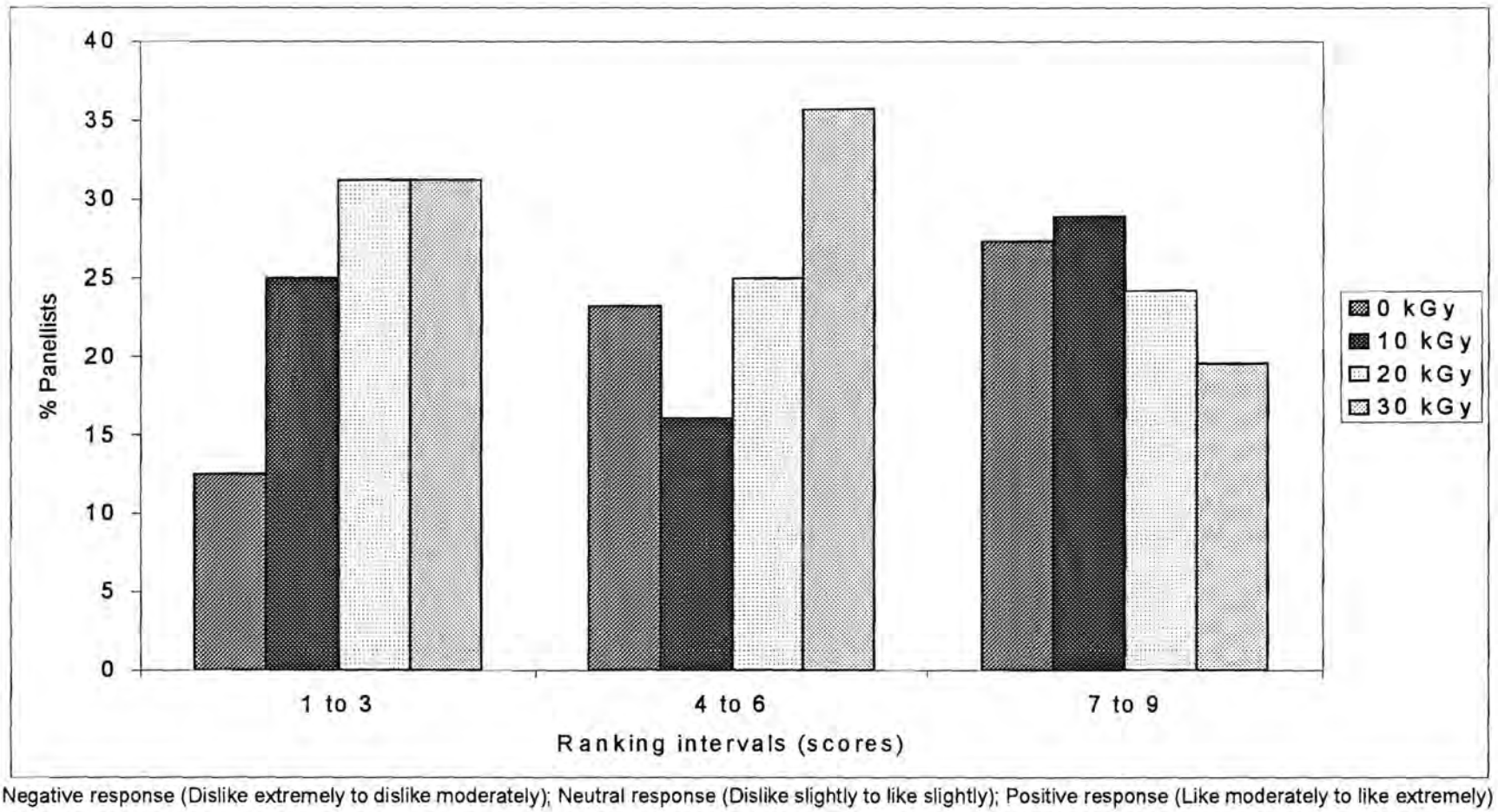
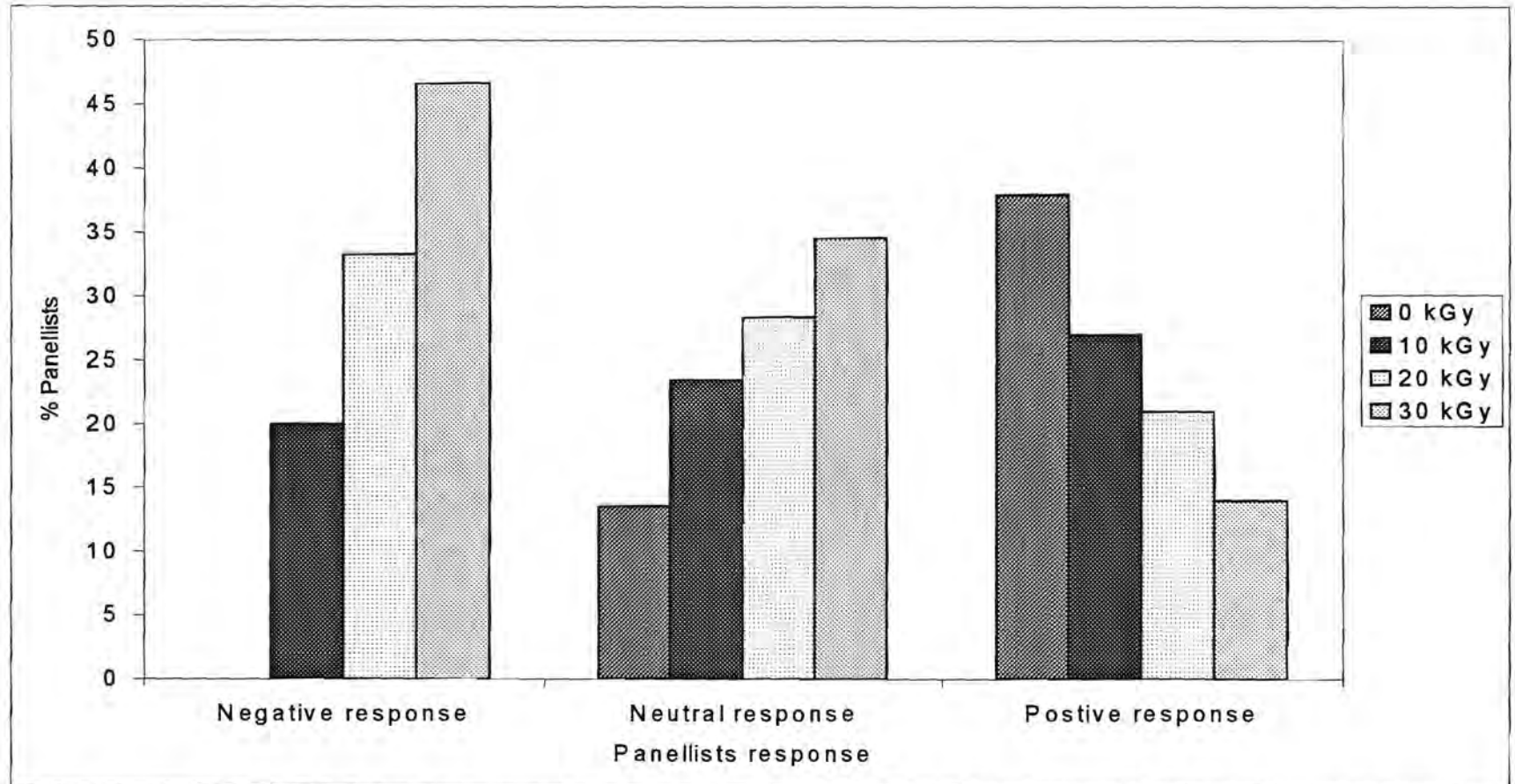


Figure 7 Percentage of positive, neutral and negative responses for the taste of the spinach relish component of the RTE meal irradiated at different doses



Negative response (Dislike extremely to dislike moderately); Neutral response (Dislike slightly to like slightly); Positive response (Like moderately to like extremely)

Figure 8 Percentage of positive, neutral and negative responses for the overall acceptability of the RTE meal irradiated at different doses

5.1.2 Effect of blanching, washing and cooking on the survival of *C. sporogenes* spores

The effects of pre-processing treatments on the survival of the *C. sporogenes* inoculated in the spinach and sorghum porridge components is shown in Table 3. Analysis of variance revealed that there was a significant decrease in the spores surviving ($1.55 \log_{10} \text{ cfu/g}$) in spinach washed with 250 mg.l^{-1} NaOCl. No significant decrease of *C. sporogenes* was observed after blanching in two water changes (at 77°C for 7 min), while significantly more *C. sporogenes* survived cooking than washing and blanching. With sorghum porridge, a significant decrease ($1.70 \log_{10} \text{ cfu/g}$) in the survival of the inoculated *C. sporogenes* was observed after cooking.

Table 3 Effect of pre processing of spinach and sorghum porridge on the survival of *C. sporogenes* ($\log_{10} \text{ cfu/g}$)

Treatment	Spinach ¹	Porridge ¹
Initial spore inoculation	4.96 ^c (± 0.15) ²	4.72 ^b (± 0.21)
Washing (Chlorinated H ₂ O)	3.41 ^a (± 0.28)	N/A
Blanching	3.09 ^a (± 0.24)	N/A
Cooking	4.03 ^b (± 0.40)	3.02 ^a (± 0.22)

1 Mean values in the same column with different letters differ significantly from each other ($p < 0.05$)

2 Standard deviations are given in brackets

N/A: Not applicable

5.2 Main experiment

5.2.1 *Effect of different nitrite levels on the survival of inoculated C. sporogenes in the ready-to-eat spinach meal*

Table 4 shows the effect of different sodium nitrite levels on the survival of the inoculated *C. sporogenes* and their subsequent growth in the spinach component of the meal stored at 10 °C for 12 d period. Statistical analysis showed that overall, there was a significant decrease ($p < 0.05$) in the *C. sporogenes* counts with increased sodium nitrite concentration. Overall, the counts were reduced by 0.8 log cycle after addition of 50 mg.kg⁻¹ of sodium nitrite and were reduced up to 1.56 log cycle when the sample was treated with 200 mg.kg⁻¹ sodium nitrite. However, there was no significant decrease in the counts over time.

Table 4 Effect of different nitrite levels to a target dose of 10 kGy on the survival of the inoculated *C. sporogenes* (\log_{10} cfu/g) in the spinach relish component of the meal stored at 10⁰C for 12 days

Time (days)	Added nitrite levels (mg.kg ⁻¹)					Time effect ¹
	0	50	100	150	200	
1	4.11 (±0.27) ³	4.06 (±0.13)	4.06 (±0.20)	2.29 (±0.14)	2.89 (±0.10)	3.89 ^a (±0.50)
6	5.01 (±0.39)	3.68 (±0.34)	3.31 (±0.09)	3.21 (±0.08)	3.25 (±0.10)	3.69 ^a (±0.73)
12	4.51 (±0.33)	3.48 (±0.15)	3.49 (±0.29)	3.14 (±0.80)	2.82 (±0.49)	3.49 ^a (±0.72)
Treatment effect ²	4.54 ^d (±0.49)	3.74 ^c (±0.33)	3.62 ^{bc} (±0.38)	3.35 ^b (±0.51)	2.98 ^a (±0.34)	

- 1 Mean values in the same column with different letters differ significantly from each other ($p < 0.05$)
- 2 Mean values in the same row with different letters differ significantly from each other ($p < 0.05$)
- 3 Standard deviations are given in brackets

5.2.2 Effect of different nitrite levels on the survival of the inoculated *C. sporogenes* in the sorghum porridge component of the meal

Table 5 shows the effect of different sodium nitrite levels on the survival of the inoculated *C. sporogenes* spores and their subsequent growth in the sorghum porridge component of the meal stored at 10⁰C for a period of 12 d. Overall, there was a significant decrease ($p < 0.05$) in clostridia count for samples treated with 0 and 50 mg.kg⁻¹. The two samples also differed significantly from the other samples. However, overall there was no significant difference between the other three samples. The overall time effect showed that there was no significant increase ($p > 0.05$) in clostridia

count between day 1 and day 6, but there was a significant increase of a 1 log cycle in clostridia counts after 12 d of storage.

Table 5 Effect of different nitrite levels to a target dose of 10 kGy on the survival of the inoculated *C. sporogenes* (\log_{10} cfu/g) in the sorghum porridge component of the meal stored at 10 °C for 12 d

Time (days)	Added nitrite levels (mg.kg ⁻¹)					Time effect ¹
	0	50	100	150	200	
1	2.97 (±0.17) ³	2.66 (±0.19)	2.52 (±0.26)	2.50 (±0.20)	2.86 (±0.60)	2.70 ^a (±0.36)
6	4.78 (±0.43)	2.93 (±0.20)	2.93 (±0.20)	2.62 (±0.37)	2.47 (±0.31)	3.10 ^a (±0.93)
12	5.45 (±0.67)	4.91 (±0.40)	2.52 (±0.26)	3.20 (±0.37)	2.97 (±0.43)	4.03 ^b (±1.07)
Treatment effect ²	4.40 ^c (±1.16)	3.50 ^b (±1.07)	2.95 ^a (±0.59)	2.77 ^a (±0.44)	2.77 ^a (±0.49)	

- 1 Mean values in the same column with different letters differ significantly from each other ($p < 0.05$)
- 2 Mean values in the same row with different letters differ significantly from each other ($p < 0.05$)
- 3 Standard deviations are given in brackets

5.2.3 Effect of cooking alone, and in combination with irradiation on residual nitrite levels

Results on the effect of cooking alone, and the effect of cooking and irradiation on the residual sodium nitrite level in the RTE spinach based relish and sorghum porridge meal are showed in Tables 6 and 7. Cooking reduced the nitrite level significantly ($p < 0.05$) in both the spinach relish and the sorghum porridge components. A decrease in nitrite levels of between 85% to 92% was

observed after cooking spinach (Table 6), while a decrease of between 62% to 75% was observed in sorghum porridge (Table 7) after cooking.

The residual nitrite level after cooking and irradiation was 355-510 % higher than after cooking alone (Table 6). In the case of the sorghum porridge, the residual nitrite level was virtually the same after cooking and after cooking and irradiation (Table 7).

Table 6 Effect of cooking alone, and in combination with irradiation to a target dose of 10 kGy on the residual nitrite levels (mg.kg^{-1}) in spinach relish

Treatments	Initial (added) nitrite concentration (mg.kg^{-1}) ¹			
	50	100	150	200
After cooking	7.56 ^a (± 1.14)	9.67 ^a (± 1.93)	13.48 ^a (± 1.68)	15.65 ^a (± 1.68)
After cooking and irradiation	40.14 ^b (± 4.08)	59.06 ^b (± 22.95)	67.30 ^b (± 25.68)	71.22 ^b (± 27.74)

- 1 Mean values in the same column with different letters differ significantly from each other ($p < 0.05$)
- 2 Standard deviations are given in brackets

Table 7 Effect of cooking alone and in combination with irradiation to a target dose of 10 kGy on the residual nitrite levels (mg.kg^{-1}) in sorghum porridge

Treatments	Initial (added) nitrite concentration (mg.kg^{-1}) ¹			
	50	100	150	200
After cooking	19.20 ^a (±1.14)	35.38 ^a (±1.93)	38.47 ^a (±5.30)	49.32 ^a (±8.14)
After cooking and irradiation	19.59 ^a (±1.02)	29.89 ^a (±5.09)	39.37 ^a (±9.60)	52.00 ^a (±10.24)

- 1 Mean values in the same column with different letters differ significantly from each other ($p < 0.05$)
- 2 Standard deviations are given in brackets

5.2.4 Effect of cooking, addition of nitrite and irradiation on the survival of the inoculated *C. sporogenes* spores in the ready-to-eat spinach relish and sorghum porridge meal

Tables 8 and 9 show the effect of cooking, addition of nitrite and irradiation on the survival of the inoculated *C. sporogenes* spores in the components of the ready-to-eat meal. Analysis showed that in all treatments there was no detectable levels ($<10 \text{ cfu/g}$) of *C. sporogenes* left in the spinach relish and sorghum porridge components of the ready-to-eat meal. No significant increase ($p < 0.05$) in clostridia count was observed with time in the sorghum porridge (Table 9). However, there was a significant increase ($p < 0.05$) in the count in the spinach relish component of the meal on day 6 for the sample treated with 0 mg.kg^{-1} nitrite and on day 12 for the samples treated with 150 and 200 mg.kg^{-1} nitrite (Table 8).

Table 8 Effect of cooking, addition of nitrite and irradiation to a target dose of 10 kGy on the survival of the inoculated *C. sporogenes* (cfu/g) in spinach relish stored at 10 °C for 12 d ⁽¹⁾

Time (days)	Added nitrite levels (mg.kg ⁻¹)				
	0	50	100	150	200
1	<10	<10	<10	<10	<10
6	415	<10	<10	<10	<10
12	<10	<10	<10	355	505

1 Appendix 5 shows the results for replicates 1 and 4 (pooled)(doses received by samples: 13.8 and 12.3 kGy) as well as for replicates 2 and 3 (pooled)(doses received by samples: 10.4 and 10.0 kGy)

Table 9 Effect of cooking, addition of nitrite and irradiation to a target dose of 10 kGy on the survival of the inoculated *C. sporogenes* (cfu/g) in sorghum porridge stored at 10 °C for 12 d

Time (days)	Added nitrite levels (mg.kg ⁻¹)				
	0	50	100	150	200
1	<10	<10	<10	<10	<10
6	<10	<10	<10	<10	<10
12	<10	<10	<10	<10	<10

CHAPTER 6

DISCUSSION

In preliminary experiments a consumer acceptability test was performed in order to obtain a cut-off point for the irradiation dose that could be used to produce a ready-to-eat spinach relish and sorghum porridge meal. Although the consumer overall acceptability test results showed that the ready-to-eat samples irradiated at 10 kGy was not as acceptable as the control sample, the two components of the meal remained acceptable up to a dose of 10 kGy. However, the limiting factor for using higher doses was the porridge component. The tasting scores for samples irradiated at 20 and 30 kGy were lower (less acceptable) than the control and 10 kGy samples in all attributes. This was attributed to the fact that there was a decrease in the sensory acceptability perceived by panellists in samples with increased irradiation dose. The decrease in the overall acceptability of the ready-to-eat meal perceived by the panelists was attributed to the loss in product appearance, texture and taste with increased irradiation dose.

The loss in appearance observed by the participants in the two components of the meal could be due to the oxidation, which might have occurred during the irradiation process. During irradiation in the presence of oxygen highly oxidising free radicals may be formed (Thakur & Singh, 1994), and this may oxidise the product which might result in the discolouration of the meal components, thereby affecting the product appearance. According to Swallow (1991) there is a change in the water holding capacity (WHC) when food is irradiated. This could affect the appearance of the samples irradiated at 20 and 30 kGy. Some of the panellists reported the sorghum porridge component irradiated at 20 and 30 kGy to be watery.

The textural changes of the two components of the meal with increased irradiation doses reduced the overall acceptability of the RTE meal irradiated at 20 and 30 kGy. Samples irradiated at 30 kGy were perceived to be softer than those irradiated at lower doses. The softer texture of the porridge samples after irradiation was due to the fact that irradiation results in the breakdown of complex carbohydrate molecules into simpler compounds such as dextrin, glucose, maltose and other radiolytic products (Diehl, 1990). This tendency of causing depolymerisation of the starch molecules could be the cause of softness of porridge perceived by panelists at high irradiation dose levels.

The change in texture that was observed in the spinach component with increased irradiation dose was expected although the scores recorded by panelists were still at acceptable levels for all samples. The textural loss could be due to the fact that softening of plant material usually occurs during heat treatment and irradiation processing. Heat processing damages or destroys membranes and disrupts intercellular structures, resulting in cell separations (Urbain, 1986). In addition, during irradiation processing there is a partial degradation of pectins and cellulose, which weakens the structural tissue resulting in softening or loss of texture (Ley, 1983; Urbain, 1986).

The fact that the spinach component was perceived to be more liked than the porridge component after irradiation could be due the fact that vegetables tend to lose their texture during the cooking processes. This could be the reason for the failure of the participants to detect the textural changes after irradiation in the spinach component.

Regarding the taste of the two components of the meal, irradiation had less effect on the taste of the spinach than on the sorghum porridge. The possible explanation for this could be due to undesirable changes that occurs in certain foods, which may be caused directly by irradiation or indirectly as a result of

post irradiation reactions (Jay, 1998). Water undergoes radiolysis when irradiated and in addition, free radicals are formed and react with each other as diffusion occurs. Some of the products formed along the track escape and then react with solute molecules. In presence of oxygen the oxidation is accelerated (Thakur & Singh, 1994) and the formation of off-flavours and off-odours are promoted due to formation of peroxides during the oxidation process (Urbain, 1989).

Also, proteins and other nitrogenous compounds are the most sensitive to irradiation effects in foods with regard to off flavour development. The products of irradiation that have been reported are NH_3 , hydrogen, CO_2 , H_2S , amides and carbonyls. Apart from other factors, the formation of these products depends on the irradiation dose, temperature, amount of oxygen and amount of moisture present. These products could influence the formation of off-flavours and off-odours.

The fact that sorghum porridge has more amino acids and peptides than spinach could be the reason for the differences in the loss of the taste of the two meal components. In addition, the depolymerisation of starch molecules that occurs in porridge could affect the taste of the porridge due to formation of glucose, maltose, and radiolytic products (Diehl, 1990; Thakur & Singh, 1994). Formation of these sugars increases the sweetness of the porridge, which is not the typical taste of the sorghum porridge. Sweetness of the sorghum porridge component of the meal was reported by panelists and this could have resulted in the low acceptability of the samples irradiated at higher doses.

Pre-processing (washing, blanching and cooking) steps were followed in order to reduce the initial spore counts before further processing. This was based on the fact that raw spinach could potentially contain high initial spore counts because it is normally grown in soil.

Sodium hypochlorite (250 mg.l^{-1}) was used because of the effectiveness of the chlorine compound to bacterial spores (Foegeding, 1983; Brackett, 1993). It has been suggested that the concentrations of chlorine normally applied to wash water can range from 5 to 250 mg.l^{-1} . However, the concentration of chlorine available to kill microorganisms can be much less. This is because chlorine is very reactive and breaks down quickly in the presence of organic matter (Brackett, 1993).

The significant decrease in the number of spores surviving after washing spinach with 250 mg.l^{-1} NaOCl could be attributed to the sporicidal effect of the chlorine compound (Hugo & Russell, 1982). Foegeding & Busta (1983b) explained that the spore coat appears to be both a target of chlorine action and a barrier to chlorine permeability and this can be removed by hypochlorite treated solution. Removal of coat proteins affects the spore viability and also may injure the surviving bacterial spore thereby increasing the lethal effect of hypochlorite on *Clostridium* spores. This could be the possible explanation for the 1.55 log reduction in spore number after washing.

Blanching (in two water changes) did not significantly reduce the number of *C. sporogenes* spores inoculated in spinach. These results were not expected. During chlorine wash the sublethal effect of the chlorine compound may injure bacteria spores, therefore these injured spores were expected to be susceptible to the mild heat treatment such as blanching. However, this postulate was not supported by other available findings (Waites *et al.*, 1977; Foegeding & Busta, 1983a). Foegeding & Busta (1983a) found that pretreatment of spores with sublethal concentrations of chlorine may increase spore heat and chemical sensitivity. Waites *et al.* (1977) postulated that chlorine treatment may alter the spore coat and cortex, thereby increasing the susceptibility to mild heat treatment.

The possible reason for blanching being ineffective after chlorine wash could be that *C. sporogenes* spores that survived the chlorine wash at the concentrations used were possibly resistant to blanching at 77 °C for 6 min. Obilana (1998) also found that blanching after a chlorine wash to be ineffective in reducing the initial microbial load in spinach. As bacterial spores are more resistant than a range of vegetative bacterial cells (Banwart, 1989; Murano, 1995a) one would expect to get similar results with spore counts.

Significantly more spores survived cooking of the spinach than cooking of the sorghum porridge. The greater survival of spores after cooking spinach could be due to spore activation by heat during cooking, which triggers their germination and outgrowth (Driessen, 1992; Anderson, Ronner & Granum, 1995). Heat shock promotes spore germination and the outgrowth of vegetative cells from them. The vegetative cells, of course, are easily destroyed by heat, radiation, and other treatments. However, the process of spore germination takes some time, and it does not occur while lethal temperatures are surrounding the spore. Because sorghum porridge takes a longer period to cool than spinach, there is a possibility that the vegetative cells that have germinated during cooking of the sorghum porridge were inactivated or were heat-injured due to the long exposure of vegetative cells to heat and this could affect their viability and therefore prevent their germination.

In both components of the meal, the significant decrease in spore count with increased sodium nitrite levels was expected. The higher levels of added sodium nitrite, which resulted in lower *C. sporogenes* counts, could be due to the ability of the nitrite to inhibit *Clostridium* species. Different authors suggested that nitrite inhibits bacteria by interfering with the metabolic systems (Woods & Wood, 1982; Jay, 1992). The present study found that there was a significant decrease in the spore counts with increased nitrite levels. This could probably rely on the fact that more nitrite was available for inhibition at higher levels than at lower levels.

With regard to the spinach relish components of the meal, there was no significant increase of clostridia counts with time. This could be due to the amount of nitrate available in spinach. Under certain conditions nitrate can be reduced to nitrite and thereby this could reduce clostridia counts with time because of the inhibitory effect of the nitrite.

However, the increased inhibitory effect of nitrite when heated could be the other possible reason for the lower counts observed in the spinach component. According to Jay (1992), Perigo *et al.* (1967) found that heating of a medium with nitrite produced a substance or agent about ten times more inhibitory than nitrite alone. This agent is referred as the Perigo-type factor. It appears that the formation of these inhibitors is due to involvement of nitrite, iron and sulfhydryl groups (Tompkin, 1993). Nitrite and iron are more available in spinach than in sorghum grain. The lower level of these substances in sorghum porridge could be the possible reason for the significant increase of the *C. sporogenes* counts in the sorghum porridge components of the meal after 12 d of storage at 10 °C, which was not observed in the spinach. In addition to the Perigo factor, porridge is more likely than the spinach relish to have developed pronounced internal anaerobiosis due to the cooking. Heat promotes the expulsion of air, and the thickness of the porridge would likely make it an ideal medium for later growth of anaerobic clostridia in microenvironments within the porridge, even in the presence of outside air.

Significantly ($p < 0.05$) lower levels of nitrite than that initially added were found in both components of the meal after cooking. The decrease in final nitrite levels may be attributed to leaching during the cooking process because of its very high solubility. However, there is also a possibility that nitrite might have changed to another form and evaporated with water. The results obtained in this experiment were similar to those of Abo Bakr, El-Iraqi &

Huissen (1986). They found a 40.9 % decrease of nitrite levels in jew's mallow after cooking. The differences in percentage decrease found in this research (85 % to 92% in spinach and 62 % to 75 % in the porridge) to that of Abo Bakr *et al.* (1986) could be due to the differences in the initial levels of nitrite in the two products. According to Abo Bakr *et al.* (1986), Hata & Ogotta (1971) showed that the nitrate and nitrite contents of potatoes were heat stable during cooking but losses occurred due to leaching from potato tissues into the cooking water. However, the higher levels of nitrite observed in the sorghum porridge as compared to those in the spinach could be due to the possible transformation of nitrite into other forms and the formation of complexes with other substances present in spinach during cooking of the spinach.

Irradiation after cooking significantly increased the residual nitrite level in the spinach relish component of the meal. These results could have a similar explanation to that of Mondy, Koushik & Munshi (1992) who reported that an irradiation dose of 1.0 kGy resulted in an increase in nitrite nitrogen concentration of potato tubers with some values being 300 % greater than the controls. The nitrite nitrogen content significantly increased with increased irradiation dosage (Mondy & Koushik, 1990). Similar results were found in the present research for samples, which received relatively higher doses from the target dose in the main experiment. During irradiation in the presence of oxygen, highly oxidising free radicals may be formed (Thakur & Sigh, 1994) and these may oxidise nitrogen from sources such as protein and nucleic acids leading to an increase in nitrate (Duodu *et al.*, 1999). Possibly the same mechanisms could result in an increase in the nitrite levels in the spinach relish component of the meal. There is also a possibility that some of the nitrate present in the spinach relish component of the meal was reduced to nitrite during the irradiation process. The smaller increase in nitrite observed in the sorghum porridge component of the meal could be due to lower nitrate levels in cereal grains than that found in vegetables. According to Abo Bakr *et*

al. (1986) the amount of nitrate present can be considered as the index of the amount of nitrite which may be formed during processing.

Nitrite in combination with irradiation reduced the *C. sporogenes* counts in both components of the meal to less than 10 cfu/g immediately after irradiation. However, the significant growth observed on day 6 in spinach treated with 0 mg.kg⁻¹ nitrite and irradiated at 10 kGy, and in spinach treated with 150 and 200 mg.kg⁻¹ nitrite on day 12, was not expected. This is because the spores at these nitrite levels were probably still viable due to the low level of nitrite in spinach after cooking (200 mg.kg⁻¹ residual nitrite is allowed in meat products in South Africa). The mild heat treatment and irradiation treatment (10 kGy) alone are insufficient to produce a safe product in case of high initial spore counts. It was noted that growth was only observed in replicates 2 and 3, where samples received irradiation doses of 10.4 and 10.0 kGy respectively, but not in replicates 1 and 4, where samples received irradiation doses of 13.8 and 12.3 kGy respectively. However, the distribution of radiants and the sample position during irradiation process could also have affected the killing effect of spores.

The reduction of the *C. sporogenes* counts in the components of the meal to less than 10 cfu/g could be due to the low initial spore counts after cooking; i.e. 4.11 and 2.97 log₁₀ cfu/g in spinach relish and sorghum porridge respectively. According to Obilana (1998) the gamma D₁₀-value of *C. sporogenes* (different isolates) in this RTE meal was found to be between 2.58 and 2.60. Therefore, a target dose of 10 kGy would result in about 4 log₁₀ cycle reduction in *C. sporogenes* counts. Thus, with the initial spore counts of about 4 log₁₀cfu/g before irradiation, one would expect less than 10 cfu/g remaining after irradiation.

The possible roles suggested for nitrites are that nitrite increases the germination rates of spores that survived heating (Pivnick *et al.*, 1970) and also potentiate the direct killing effect of the vegetative cells thereby reducing clostridia counts. According to the Task Force Report (1989), bacterial spores are more resistant to the lethal action of ionising radiation than their corresponding vegetative cells by a factor of about 5 to 15. This could mean that the D_{10} -value of the vegetative *C. sporogenes* cells is less than 2.58 and 2.26 kGy. Therefore, if nitrite induced germination, more cells would be inactivated during irradiation. This could be the possible reason for the lower counts (< 10 cfu/g) in both components of the meal after irradiation.

The formation of sugar acids and keto sugars increases when starch is irradiated in the presence of oxygen. The formation of acids leads to a decrease in the pH of the irradiated sugar solutions, which can prevent the outgrowth of the bacteria spores (Schubert, 1967, according to Diehl, 1990). This could be the reason for the lower counts (<10 cfu/g) in the sorghum porridge components of the meal in all treatments. Erasmus (1996) also found a significant decrease in pH after irradiating the starch suspension. The pH decreased from 7.43 to 6.61 after irradiation. The decrease in pH was probably due to formation of free fatty acids and formic acid.

Although samples were irradiated at a target dose of 10 kGy they received relatively higher doses (Section 4.2.1.3). Samples irradiated at 13.8 and 12.2 kGy did not show detectable clostridia growth, whereas growth was observed in some of the samples that received a dose of 10.4 and 10.0 kGy. The reason that samples receiving higher doses had less than 10 cfu/g could be due to the fact that, apart from the direct killing effect of the higher irradiation doses, higher amounts of nitrite were being produced. Higher levels of nitrite could increase the germination rates of spores that survived and these rapidly germinating spores were then inhibited by nitrite and subsequently died before nitrite levels decreased appreciably (Pivnick *et al.*,

1970). However, the growth observed in spinach could be due to improper distribution of the irradiation during the irradiation process.

Although no literature was available on the naturally occurring spore 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 & Rodehamel, 1990). According to Odlug & Pflug (1978) the National Canners Association found 10^2 to 10^3 bacterial spores per gram of harvested tomatoes. They suggested that *C. botulinum* spores would only contribute a fraction of the total spores present. From that statement, Obilana (1998) assumed that *C. botulinum* spores occurring naturally on spinach would not be more than 10^3 spores per gram.

In the present study with the initial *C. sporogenes* spore count of $4.96 \log_{10}$ cfu/g in spinach and $4.72 \log_{10}$ cfu/g in porridge, it was found that the pre-processing alone reduced the *C. sporogenes* counts with 0.93 log cycle in spinach and 1.70 log cycles in porridge. Nitrite alone reduced the counts with 1.19 and 1.56 log cycles in samples treated with 150 and 200 mg.kg⁻¹ respectively and, 0.8 and 0.92 log cycle in samples treated with 50 and 100 mg.kg⁻¹ respectively in the spinach relish component. In the sorghum porridge component, there was a 0.90 log cycle reduction in samples treated with 50 mg.kg⁻¹ of nitrite, whereas 1.45, 1.63 and 1.63 log cycles reduction occurred in samples treated with 100, 150 and 200 mg.kg⁻¹ of nitrite respectively. Furthermore, a target dose of 10 kGy could reduce counts by about 3.88 log cycles (Obilana, 1998). These results could suggest that with nitrite and irradiation combination processes about 4.68 log cycles reduction could be expected in the spinach component and about 4.78 log cycles in the sorghum porridge component of samples that had been treated with at least 50 mg.kg⁻¹ of nitrite and irradiated at 10 kGy.

The nitrite in combination with irradiation reduced the counts to undetectable levels for at least 6 d in both components of the meal regardless of the nitrite levels. This suggests that if the meal had undergone pre-processing, lower initial levels of spores could be expected and the combination treatment would reduce the counts at least 4 log cycles. Therefore, a safe ready-to-eat meal could be possibly expected when a pre-processing followed by a combination treatment of at least 50 mg.kg⁻¹ of nitrite and a target dose of 10 kGy of irradiation had been applied. However, this would depend on the absence of any further contamination of the meal.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

Application of a 10 kGy target irradiation dose was shown to produce a ready-to-eat spinach relish and sorghum porridge meal of acceptable sensory qualities. Irradiating at higher doses affected the sensory acceptability of the meal components, especially the sorghum porridge component of the meal.

Pre-processing steps are essential for reducing the initial spore load of the raw materials of the ready-to-eat meal components. In the present work pre-processing steps reduced the clostridia counts about 0.93 log cycles in spinach and 1.70 log cycles in the sorghum porridge. In spinach, washing in chlorinated water ($250 \text{ mg.l}^{-1} \text{ NaOCl}$) resulted in about a 1.55 log reduction of the clostridia count. However, blanching had no effect and there was a significant increase of clostridia count after cooking because of heat-induced massive germination of spore. The approximate 1.70 log cycle reduction in the counts in the sorghum porridge could be due to long exposure of vegetative cells to heat.

Sodium nitrite alone resulted in decreased clostridial counts with increased concentration. However, an increase in counts in the sorghum porridge component of the meal was observed after 12 d.

In both components of the meal, cooking reduced the nitrite levels. However, there was an increase in the nitrite levels in the spinach relish component of the meal after irradiation. This was probably because of the oxidation of nitrogen sources such as protein and nucleic acids.

Combination of nitrite and irradiation was used in the present research in order to reduce the use of severe irradiation treatments. This reduced the *C. sporogenes* counts in both components of the meal to less than 10 cfu/g immediately after processing. However, growth was unexpectedly observed in the spinach relish component of the meal after day 6 and 12 in samples treated with 0, and 150 and 200 mg.kg⁻¹ of nitrite respectively. This could be due the fact that a mild heat treatment and irradiation treatment of 10 kGy alone is insufficient to produce a safe product if the initial spore count is high. However, the distribution of radiants and the sample position during irradiation process could also have affected the killing effect of spores. Nevertheless, this could mean that the combination treatment of at least 50 mg.kg⁻¹ of nitrite and irradiation at a target dose of 10 kGy could be applied to produce a ready-to-eat meal with a shelf life of at least 6 d provided there is no post processing contamination of the meal.

The use of higher levels of sodium nitrite in combination with irradiation are recommended for processing spinach relish and sorghum porridge meal components in order to produce a safe ready-to-eat meal with a longer shelf life. This is because a higher amount of nitrite is lost during cooking therefore lower level of nitrite is available for preservation purposes; 200 mg.kg⁻¹ residual nitrite is allowed in meat products in South Africa. However, sensory analysis would be required in order to determine the sensory acceptability of the meal after addition of higher levels of nitrite.

CHAPTER 8

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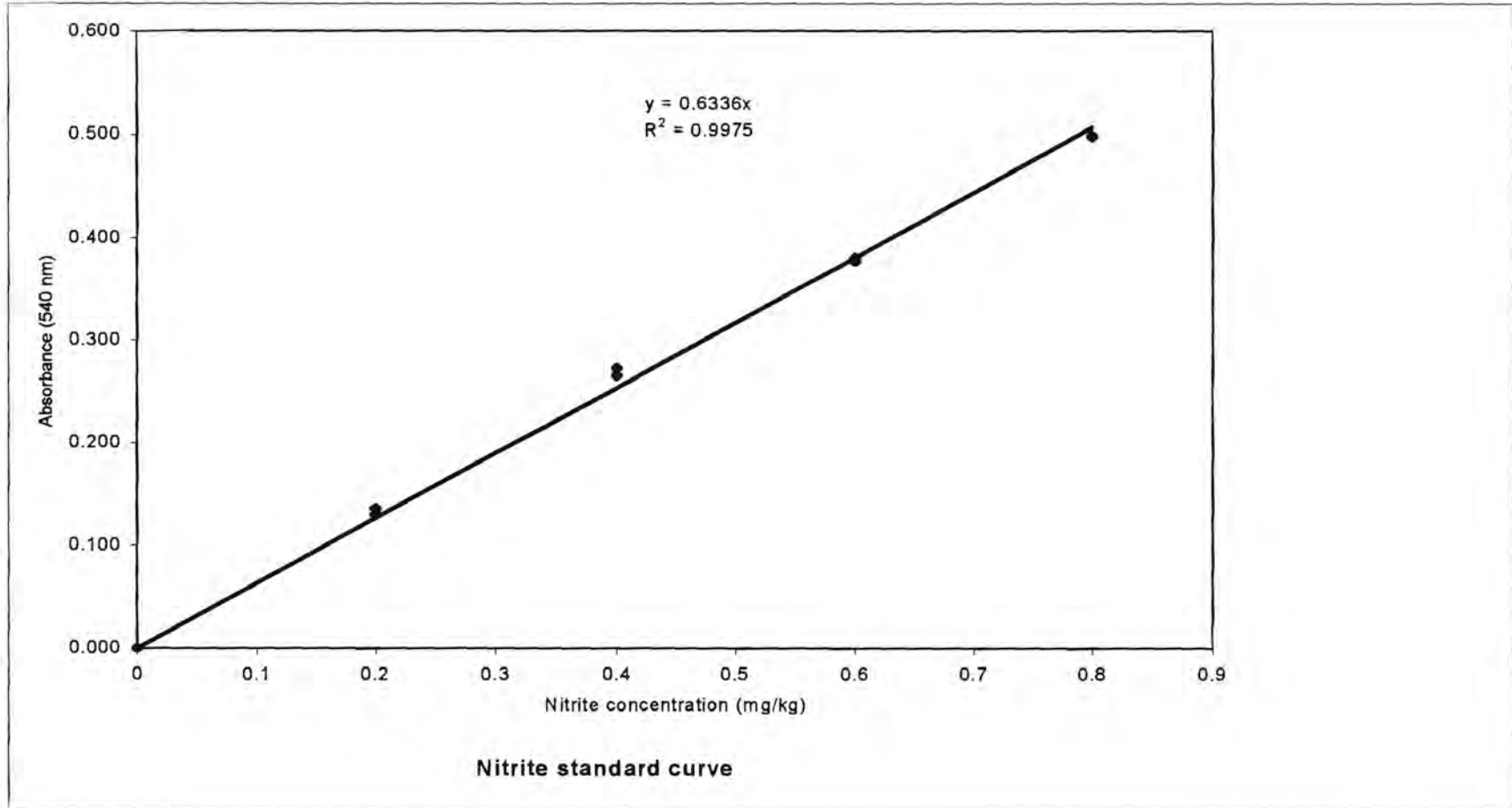
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Appendix 1

Sodium nitrite standard curve





Appendix 2

An evaluation form for the consumer acceptability test



CONSUMER EVALUATION OF SORGHUM PORRIDGE AND SPINACH RELISH MEAL

Name : _____
Age : _____
Sex : _____
Date : _____

You are provided with four samples of sorghum porridge and spinach relish meal, which you are required to evaluate their sensory characteristics in terms of their appearance, texture, taste, and overall acceptability. You may eat the porridge together with spinach, as you would normally do when you eat your meal. Please, rinse your mouth with water before starting tasting and in between before tasting each sample.

For each sample of meal, please write down how much you like the appearance, texture (how you feel in your mouth), taste, and the overall acceptability of the sample by marking the appropriate block on each scale with a [X].



Sensory evaluation form

Code:

	Appearance			Texture			Taste			Overall acceptability		
Like extremely												
Like very much												
Like moderate												
Like slightly												
Neither like nor dislike												
Dislike slightly												
Dislike moderately												
Dislike very much												
Dislike extremely												

Comments: _____

Thank you for taking your time to participate in this consumer sensory evaluation of a Ready-to-Eat (RTE) sorghum porridge and spinach relish meal.



Appendix 3

Master Sheet: 9 Point hedonic scale for the sensory acceptability test

MASTER SHEET: 9 POINT HEDONIC SCALE

A = 1

B = 2

C = 3

D = 4

Panellist	Perm.	Serving	Treat.	Code	Appearance	Texture	Taste	Overall accept.
1	4	1	D	915				
	1	2	A	186				
	3	3	C	039				
	2	4	B	976				
2	1	1	A	522				
	4	2	D	660				
	2	3	B	187				
	3	4	C	608				
3	3	1	C	668				
	4	2	D	514				
	1	3	A	104				
	2	4	B	230				
4	4	1	D	589				
	3	2	C	163				
	2	3	B	008				
	1	4	A	085				
5	2	1	B	716				
	3	2	C	067				
	4	3	D	628				
	1	4	A	142				

Note: A = 0 kGy; B = 10 kGy; C = 20 kGy; D = 30 kGy

Hedonic point scale; 1=Dislike extremely; 2=Dislike very much; 3=Dislike moderately; 4=Dislike slightly; 5=Neither like nor dislike; 6=Like slightly; 7=Like moderate; 8=Like very much; 9=Like extremely



Appendix 4

Summary of the panellists comments on the appearance, texture, taste and the overall acceptability of the meal

Sorghum porridge

Sample	Appearance	Texture	Taste
0 kGy	Very light Much attractive Excellent	Best Nice and firm Excellent	Very nice Delicious
10 kGy	Not very bad Nice than the rest	Granules bit too fine Bit firm Good quality Bit soft	Well cooked Tasty
20 kGy	Watery Colour is acceptable Appearance is acceptable Bit watery Too white	Too soft Not firm Texture not right Slightly soft texture	Taste not good Undercooked Bit sweet Sour
30 kGy	Too watery Looks overcooked Too whitish	Too soft Soft and nice texture Need to be improved Not firm Bad texture	Did not taste like sorghum porridge Did not taste nice Moderate Taste spoiled Sweet



Appendix 5

Effect of cooking, addition of nitrite and irradiation on the survival of the inoculated *C. sporogenes* (cfu/g) in spinach relish stored at 10 °C for 12 d for replicates 1 and 4 and replicates 2 and 3 respectively

Effect of cooking, addition of nitrite and irradiation¹ on the survival of the inoculated *C. sporogenes* (cfu/g) in spinach relish stored at 10 °C for 12 d for replicates 1 and 4

Time (days)	Added nitrite levels (mg.kg ⁻¹)				
	0	50	100	150	200
1	<10	<10	<10	<10	<10
6	< 10	<10	<10	<10	<10
12	<10	<10	<10	< 10	<10

1 2.3 kGy for replicate 1
13.8 kGy for replicate 4

Effect of cooking, addition of nitrite and irradiation¹ on the survival of the inoculated *C. sporogenes* (cfu/g) in spinach relish stored at 10 °C for 12 d for replicates 2 and 3

Time (days)	Added nitrite levels (mg.kg ⁻¹)				
	0	50	100	150	200
1	<10	<10	<10	<10	<10
6	415	<10	<10	<10	<10
12	<10	<10	<10	355	505

1 10.4 kGy for replicate 2
10.0 kGy for replicate 3