SHELF LIFE EXTENSION OF GROUND BEEF USING RADURIZATION AND CONTROLLED ATMOSPHERE PACKAGING

A Thesis
Submitted to the Faculty of Graduate Studies
The University of Manitoba

by

Theodore (Ted) G. Tishinski

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1. ABSTRACT

Shelf life extension of refrigerated ground beef by irradiation has been investigated. It has been shown that refrigeration at 4°C, and the use of controlled atmosphere packaging in conjunction with irradiation at a radurization dose level of 2.5 kGy is effective in extending the shelf life of ground beef. All irradiations were carried out in evacuated dual bags (anaerobic) on ice (0-2°C) to minimize the formation for radiolytic products, particularly lipid peroxides. The dual bag packaging system consisted of an inner oxygen permeable bag surrounded by an outer oxygen impermeable barrier bag. The removal of the outer bag facilitated the reintroduction of oxygen during storage in the inner single bag by normal diffusion. Dual (anaerobic) and single (aerobic) bag storage were investigated to assess their effect on germination and outgrowth of inoculated clostridial spores to address the question of safety.

Inoculation pack studies using Clostridium sporogenes as an indicator organism for C. botulinum were conducted at refrigerated and abuse temperatures. Growth of C. botulinum is associated with toxin formation. A selective/differential assay was developed to monitor C. sporogenes in the high background microflora contained in stored radurized ground beef. Work on establishing the safety of the ground beef patties was done first, followed by

investigation into the extension of shelf life.

The safety of the product/process was demonstrated as there was no growth of inoculated C. sporogenes in radurized ground beef patties at storage temperature of less than or equal to 15°C up to four weeks of storage in either packaging condition. There was growth during storage at 22°C in both types of packaging conditions but was slightly earlier in the single bag (aerobic). However spoilage was detectable in both types of packaging conditions due to the surviving microflora, prior to the growth of C. sporogenes.

Refrigerated (4°C) shelf life extension of radurized ground beef patties was also demonstrated in this study. Patties stored in dual bag packaging had a microbiological shelf life of >24 days while single bag packaging gave acceptable product up to 16 days. Effective shelf life was limited by unacceptable patty odour to be 19 and 16 days in the dual and single bag, respectively. Colour stability was poor in both types of packaging conditions but improved by the addition of 0.1% ascorbic acid.

The radurization of fresh ground beef patties, in conjunction with controlled atmosphere packaging, can extend refrigerated shelf life, without increasing the botulism hazard even if oxygen is not reintroduced (dual bag package).

2. INTRODUCTION

The extension of shelf life in refrigerated meat products (Palmin et al., 1974; Urbain, 1983), specifically ground beef by irradiation, has been documented (Niemand et al., 1983; Tiwari and Maxcy, 1971). Irradiation dose of >2 kGy (radurization) facilitates this extension by reducing the number of spoilage organisms. An irradiation dose of >2 kGy has been shown to insure the elimination of vegetative pathogenes, such as Salmonella. In lipid rich foods radiolysis can lead to lipid peroxidation, which reduces the sensory qualities of the product. Therefore, irradiation under anaerobic conditions is desirable since this environment reduces the amount of radiolytic products formed (Nawar, 1983; Delincee, 1983).

Since the radiation D_{10} value of C. botulinum spores is much greater than that of the vegetative spoilage and pathogenic organisms (Banwart, 1979; Singh, 1987), they can survive a radurization dose, creating a safety hazard. This botulism safety concern is associated with storage in anaerobic packaging, if there is temperature abuse. The concern is that the shelf life may be extended in such a manner that detectable spoilage does not occur prior to toxin production by surviving C. botulinum.

The presence of oxygen is one of the factors that can inhibit the

germination and growth of C. botulinum. The aim of this research was to investigate the use of a dual bag packaging system to reintroduce oxygen after radurization to improve safety and to reduce lipid peroxidation. The reintroduction of oxygen after irradiation was expected to be facilitated by the removal of the gas barrier outer bag, exposing the permeable inner bag of the packaging system to air. This would result in slow diffusion of oxygen at a known rate from the permeability characteristics of the inner bag.

Inoculation pack studies using C. sporogenes spores as an indicator organism of C. botulinum were undertaken to monitor the potential botulism safety concern. The concern was that if clostridial spores were present in ground beef patties, and if the shelf life of the patties were extended by radurization (destroying vegetative pathogens), they could survive this dose level and germinate and outgrow prior to detectable spoilage if the patties were packaged in a low oxygen environment. The use of a Controlled Atmosphere Packaging (CAP) system which would allow for the irradiation of the patties in an anaerobic condition (to limit production of radiolytic products and improve subsequent patty quality) followed by the reintroduction of air into the package as an inhibitor of clostridial growth was therefore investigated.

The radurization of fresh ground beef patties, in conjunction with CAP, was investigated to determine if refrigerated shelf life could be extended, and if safety under temperature abuse conditions (up to 22 °C) could be augmented.

The bright red colour of ground beef is perceived as an important

attribute by consumers. The addition of ascorbic acid was examined as a method of improving the colour stability of ground beef patties during refrigerated storage.

3. LITERATURE REVIEW

3.1 Shelf Life Extension

The benefits of extending the shelf life of ground beef patties include reduced waste at the retail and consumer level, as well as facilitating the more economic large centralized processing, which reduces unit cost due to volume purchasing and wider distribution routes (Agriculture Canada, 1990). The extension of ground beef shelf life has been achieved by using reduced storage temperatures, modified atmosphere packaging (MAP), controlled atmosphere packaging (CAP) (Genigeorgis, 1985; Hintlian and Hotchkiss, 1986; Hotchkiss, 1988; Silliker and Wolfe, 1980), and irradiation. Shelf life may be defined as the number of days a product is safe and is deemed acceptable by the consumer, from the day of its production. A product is only acceptable if its basic quality attributes are met. Kramer and Twigg (1984) stated that the sensory qualities of concern were; appearance (colour, size, shape), kinesthetics (texture, viscosity, consistency), and flavour (odour, taste).

Hidden attributes, such as wholesomeness (nutritive value, microbiological quality) often can not be detected by the consumer but are technically important criteria for shelf life. Microbiological quality is composed of two parts; spoilage organisms which affect the odour and pathogenic organisms which give rise to

safety concerns. There is concern that if the spoilage organisms are suppressed in order to extend the shelf life, there is the possibility that pathogenic organisms may flourish with the spoilage not being detectable to the consumer. Rancidity is the flavour attribute of most concern in ground beef due to its high fat content (10-30%). Further a bright red colour is desirable in ground beef as it is associated with freshness.

The initial microflora of ground beef has been investigated by several researchers (Chambers et al., 1976; Duitschaever et al.; 1973; Kotula et al., 1987); Shoup and Oblinger, 1976; Westhoff and Feldstein, 1976). Many of these studies amongst others (Johnson et al., 1979) show that bacterial levels found in ground beef, in part, reflect the levels present on the surfaces of carcasses at the time of deboning and grinding.

The succession of microflora in aerobically packaged ground beef during refrigerated storage has been identified (Ayres et al., 1980; Rhee et al., 1985). Although pseudonmonads are the major bacteria present in ground meat, others that have been identified, in much smaller numbers, are Micrococcus species, Brochothrix thermosphacta, Cornyeform bacteria, heterofermentative Lactobacillus and Staphylococcus species (Rhee et al., 1985; Singh, 1987). The dominant spoilage organism, in ground beef is the pseudomonades group. In vacuumed packaged ground beef, Lactobacilli become the predominant bacteria on refrigerated storage (Lee et al., 1984).

The spoilage microflora during storage is affected by intrinsic (pH,

moisture content, initial microbial load, additives) and extrinsic factors (storage temperature, packaging condition, processing operations). The storage condition and microbial load of the meat trim and fat used to make ground beef after grinding affects the initial microflora (Johnson et al., 1979). The initial microbial load will in turn have an effect on the rate of microbial spoilage (Madden and Moss, 1987).

Myoglobin is the main pigment in meat. Colour intensity is a function of myoglobin can exist: reduced myoglobin (red-purple), oxymyoglobin (bright red), and met myoglobin, 1973; Seideman et al., 1984; Singh, 1987). Although these three forms generally exist in a dynamic equilibrium with each other depending on the conditions, other irreversible oxidation forms are known (Francis, 1985). The form in which myoglobin exists is determined by: oxygen tension, pH, salt concentration, ultraviolet light, temperature, carbon dioxide, and microbial growth (Rhee et al., 1985). Bala et al, (1977) showed microbe growth, with the resultant production of lipolytic and proteolytic enzymes, degraded the fat and protein in ground beef causing discolouration. In general if the oxygen concentration is <0.2%, 0.5-1.3%, or >7.9-10.5%, the pigment form is reduced myoglobin, metmyoglobin, or oxymyoglobin, respectively (Govindrajan, 1973). Work on some of the factors that affect the shelf life of ground beef are briefly reviewed below.

3.1.1 Effect of Storage Temperature

Storage of ground beef below the freezing point (frozen storage) can yield an extremely long microbiological shelf life. There are some sensory limitations because loss of moisture (thaw exudate) and freezer burn can limit the time that colour and texture are acceptable after thawing (Andersen et al., 1990; Berry and Leddy, 1989; Bhattacharya et al., 1988a; Tutuncu et al., 1990). The influence of freezing during storage in various types of packaging on lipid oxidation is also a concern (Bhattacharya et al., 1988b). There is also a consumer trend towards foods which are not perceived as fresh, compared to those that are processed. The reduction of storage temperature while still above the freezing level is an important option alone or in conjunction with other methods, in extending the shelf life of ground beef (Jaye et al., 1962; von Holy and Holzapfel, 1988). Reduced temperatures retard the growth of microorganisms present (Ayres et al., 1980; Jay, 1970) and the rate of lipid oxidation.

3.1.2 Modified Atmosphere Packaging (MAP)

MAP is the packaging of a product in a high gas barrier material, with the gas environment changed once. Oxygen (Baran et al., 1970), carbon dioxide (Bentley et al., 1989; Madden and Moss, 1987), and nitrogen (Bentley et al., 1989; Lee et al., 1984) and combinations of these gases (Bentley et al., 1989; Hess et al., 1980) have been investigated to determine their effect on shelf life of ground beef. Bentley et al. (1989) compared the shelf life of ground beef

patties packaged in CO_2, N_2 and under vacuum. They found that a shelf life (microbial and sensorial) of 7 days was possible on storage at 2°C under most of these packaging conditions. Vacuum packaging is a form of MAP with the gas environment removed from the package. Baran et al. (197), Grau (1978) and von Holy and Holzapfel (1988) have also investigated shelf life extension of ground beef by vacuum packaging. Nassos et al. (1983) showed vacuum packaging resulted in reduced pH, while lactic acid concentration and percentage of lactic acid bacteria in the microflora increased.

Duan et al. (1971) and Huffman et al. (1975) showed that the gas environment surrounding packaged meat had an effect on the colour of the meat. When using oxygen contents high enough (7.9-10.5% O_2) for bright red oxymyoglobin formation, it eventually leads to the development of the brown coloured metmyoglobin and increased lipid oxidation upon storage.

The oxidation of the lipid portion of the ground beef gives rise to rancid flavour. Increased presence of oxygen in a package over extended periods of time leads to lipid oxidation and rancid flavour. Chen et al. (1984) found that vacuum packaging reduced lipid peroxidation as measured with the 2-thiobarbituric acid test (TBA) in ground meat from beef and pork. Lynch et al. (1986) likewise found benefits ni vacuum packaged ground beef which in this case resulted in improved cooked flavour but the raw beef aroma was poorer than from ground beef packaged in oxygen permeable bags. Extended storage in vacuum packing, however, lead to decreased sensory (odour) scores due to increased lactic acid

concentrations as a result of the growth of lactic acid bacteria (Nassos et al., 1985). Oxidative rancidity in ground beef can be further reduced if carbon dioxide is included in MAP packaging as compared to just vacuum packaging or oxygen permeable packaging (Baran et al., 1970; Rhee et al., 1985).

3.1.3 Controlled Atmosphere Packaging (CAP)

CAP is the packaging of a product in a variable gas barrier material, with the gas environment changed and selectively controlled. The gas environment can be controlled by the degree of gas permeability of the packaging material or by external means such as an external gas source. Generally, external modification of the gas environment is used with bulk products such as fruits, vegetable,s or bulk meat. Individual retail or consumer packs must rely on the selection of the packaging material to control the environment since external modification is expensive. Jay et al. (1962) and Baran et al. (1970) compared the effectiveness of CAP on microbiological quality of ground beef using low and high oxygen packaging which showed that the former (low oxygen packaging condition) effectively inhibited aerobic growth. For example, Baran et al. (1970) found that while aerobic bacteria ceased to grow in low 0_2 packaging conditions by day 6, they continued to grow until day 21 in high O_2 packaging conditions.

Grau (1978), Griffin et al. (1982) and Savell et a. (1986) investigated the effect of packaging material permeability on the stability of colour in vacuum packaged beef.

Andersen et al. (1990) found lipid oxidation of ground beef in medium oxygen permeable packaging (1000 $sm^3/m^2/24$ h at 25 °C) increased with time during storage, due to exposure to oxygen, fluorescent light, and addition of salt to ground beef. Griffin et al. (1982) examined a number of sensory attribute with respect to packaging beef in films of various gas permeabilities.

3.1.4 Additives

The addition of sulphur dioxide or ascorbic acid is known to lead to extension of shelf life of meats. von Holy and Holzapfel (1988) investigated the addition of sulphur dioxide alone, and ascorbic acid in conjunction with vacuum packaging which extended microbiological shelf life of ground hamburger at low temperatures (O °C). Benedict et al. (1975), Greene et al. (1971) and Shivas et al. (1984) found that there was an increased colour stability and reduced lipid oxidation by the addition of ascorbic acid to ground beef. Modic et al. (1982) reported that the addition of salt and a mixture of citric acid and ascorbic acid extended the microbiological shelf life and the sensorial qualities of refrigerated ground beef (5 °C) by a factor of two.

Extension of the shelf life of ground beef may be accomplished by inhibiting the growth of spoilage microflora by maintaining or reducing the meat pH. Modification of pH was investigated by adding lactic acid bacteria culture (Reddy et al., 1970) and by the addition of glucose to provide a carbon source for acid production by the natural microflora (Shelef, 1977). They were able to show

an extension of shelf life of ground beef from 5 days to 8-10 days on storage at 5 °C.

3.1.5 Irradiation

Irradiation or processing with ionizing radiation is grouped into three categories based on the applied dose. The dose range designations are: radappertization (commercial sterility), >20 kGy; radicidation (destruction of all non-spore forming organisms) <10 kGy, and radurization (radiation pasteurization <10 kGy (Jay, 1970; Josephson and Peterson, 1983). The source of the ionizing radiation can be radioactive isotopes (cesium-137 or cobalt-60), or electrical sources (electron beam accelerator, or X-ray; See Table on various sources in Singh, 1991a).

3.1.5.1 Microbiology

The surviving microflora after irradiation are a function of the initial level of the microflora, and the effectiveness of the dose applied. The radiation sensitivity (generally given as a D_{10}-value which denotes the dose required for reduction of microbes by 90% of the initial load) varies among organisms. The effectiveness of the dose applied is in turn dependent on the food type, temperature, percent oxygen and water activity (Aw) at the time of irradiation (Maxcy, 1983).

Irradiation processing can extend the shelf life of ground beef by reducing

the level of spoilage organisms present (Niemand et al., 1980; Tiwari and Maxcy, 1971). It can also make a product safer by eliminating/reducing pathogenic organisms (Billon et al., 1968; Maxcy, 1983; Palumbo et al., 1986; Tarkowski et al., 1984). The radiation D_{10}-value or the degree of resistance of organisms varies with species, (Banwarf, 1979; El-Zawahry and Rowley, 1979; Lambert and Maxcy, 1984; Palumbo et al., 1986; Tarkowski et al., 1984), irradiation temperature, and gas atmosphere during irradiation (Maxcy, 1983).

As with MAP and CAP technology, there is concern that irradiation at sub-sterilization levels may be selective towards spoilage organisms, allowing spore forming pathogens to preferentially proliferate (Farkas, 1989; Maxcy, 1983).

Spoilage:

The dominant spoilage microflora of ground beef changes depending on the dose applied and the storage conditions after treatment. Maxcy (1983) reviewed the factors affecting the presence of residual organisms in food after substerilization doses. Okafor (1973) investigated various irradiation doses and storage temperatures on vacuum packaged ground beef and found that at 1 kGy dose pseudomonades and microbacterium did not survive, and at 3 kGy dose some of the lactobacilli, yeasts, streptococci, coliforms and micrococci survived. Ayres et al. (1980) also found that yeast of the genera Torulopsis, Candida, and Rhodotorula became the dominant spoilage organisms in refrigerated ground

beef when bacterial levels were reduced by pasteurizing doses of irradiation or by chemical additives such as sulphur dioxide (von Holy and Holxapfel, 1988). Johannsen et al. (1984) and Niemand et al. (1983) observed that when vacuum packaged hamburger packaged in an oxygen permeable film was irradiated with a dose of 2.4 kGy and stored at 4°C, lactic acid bacteria and yeast were found to dominate.

When using radappertization doses (>20 kGy), the highly radiation resistant vegetative surviving microflora included Moraxella acinetobacter and Micrococcus radiodurans (Bruns and Maxcy, 1979; Firstenberg-Eden et al., 1980; Ma and Maxcy, 1981). However, Snyder and Maxcy (1979) determined that the surviving M. acinetobacter cells did not grow in ground beef unless water was added to increase the a_w.

Pathogens:

Several researchers have investigated the elimination of vegetative pathogens of public health concern from ground beef using irradiation. It was concluded that a dose of 2-2.5 kGy would reduce the presence of salmonella (Maxcy, 1983; Billion and Serrano, 1968), Yersinia enterocolitica and Campylobacter jejuni (Maxcy, 1983) from the levels commonly found in ground beef to those presenting no danger to the consumer. Radurization levels of $\leq$2.5 kGy should eliminate vegetative pathogens at the contamination levels found in ground beef, while reducing but not eliminating spoilage organisms due to their

higher initial concentrations.

The D_{10}-values of Clostridium botulinum spores in ground beef were investigated by Anderson et al. (1967), Bruns and Maxcy (1978), Fernandez et al. (1969) and Grecz et al. (1971). The much higher D_{10}-value of the clostridial spores as compared to those for the spoilage organisms and vegetative pathogens means that a dose that destroys the spoilage organisms will not eliminate the most resistant clostridial spores. Thus, if low oxygen packaging conditions exist in conjunction with radurization there is a botulism safety concern about the possibility of toxin production, as with MAP packaging. In fact, even though the radappertization doses of greater than 20 kGy would reduce many of the spores they can not destroy preformed C. botulinum neurotoxin type A (Fernandez et al., 1968; Rose et al., 1988) or staphylococcal enterotoxin A in ground beef (Rose et al., 1988). Thus, proper handling of meat prior to radurization, as in any other processing method, is of paramount importance since irradiation at even high doses will not eliminate pre-existing toxins.

3.1.5.2 Colour

Richards and Morrison (1971) studied the effect of various irradiation doses and packaging conditions with refrigerated storage on the colour of fresh beef slices. The packaging conditions investigated include oxygen permeable (package 1) and barrier packaging (package 2), as well as permeable packaging overwrapped with a barrier package (package 3). The latter "dual" packaged

irradiated beef (package 3) was stored for various times at 4.4 ° C before removal of the outer overwrap.

3.1.5.3 Sensory

Irradiation off-flavour and off-odour components from ground beef (radappertized n the presence of air) were identified by Batzer et al. (1959) and Wick et al. (1967). Wick et al. (1969) narrowed down the volatile components of irradiation odour in ground beef to methional, 1-nonanal and phenylacetaldehyde (Reineccius, 1979; Singh, 1991b). Cohen et al. (1977) found slightly reduced but acceptable sensory scores for radiation sterilized vacuum packaged ground beef. Niemand et al. (1980) reported that in vacuum packaged, radurized (up to 3 kGy at 25 ° C) ground beef the sensory panel was able to detect samples irradiated at does higher than 2 kGy but did not find them to be objectionable. Thus, keeping irradiation doses to a minimum is important for maintaining good sensory attributes.

3.2 Microbiological Safety

The microbiological safety concern can be summarized as follows: if the spoilage organisms are suppressed in order to extend shelf life, there is the possibility that pathogenic organisms may flourish with no detect spoilage. Inoculation pack studies, i.e. the inoculation with the pathogen(s) of concern into

the food studies and its storage under controlled abuse conditions (at elevated temperature) are the common investigative research methods to determine the safety of a process. Several workers have recently reviewed the safety concerns associated with CAP/MAP (Genigeorgis, 1985; Hintlian and Hotchkiss, 1986; Hotchkiss, 1988; Silliker and Wolfe, 1980). The vegetative pathogens which present potential hazard in meat are; Salmonella, Staphylococcus, and Enterococci, while the spore former of concern are the proteolytic strains of C. botulinum.

The microbiological safety concerns of radurized and other sub-sterilization doses irradiated foods have been reviewed (CAST, 1987; Farkas, 1989; Maxcy, 1983). These reviews indicate that radiation processing that will eradicate salmonellae will also effectively eliminate less resistant vegetative pathogens. These include Enterobacteriaceae, Y. enterocolitica, Vibrio, A. hydrphila, C. jejuni, Shigella spp., and Listeria monocytogenes. Thus a radurization dose of >2 kGy, which will essentially eliminate Salmonella from ground beef (Maxcy, 1983), would also destroy the aforementioned vegetative pathogens (Maxcy, 1983).

3.2.1 C. botulinum

With the use of reduced oxygen levels in MAP/CAP there is added concern that if present, the toxin producing anaerobic spore former, C. botulinum, may render a packaged food toxic (botulism poisoning). The factors affecting the

growth and toxin production of C. botulinum, have been reviewed (Hauschild, 1989; Hintlian, 1986; Silliker and Wolfe, 1980; Sperber, 1982). These factors include: temperature, pH, a_w, substrate, additives, oxidation reduction potential (E_h), and headspace gas composition and competing microflora. Production of toxin by C. botulinum is associated with spore germination, and outgrowth of vegetative cells (Duda and Slack, 1969; Siegel and Metzger, 1979).

In inoculation pack studies to determine safety, work has been done to monitor the actual formation of toxin (Baker and Genigeorgis, 1990; Pelroy et al., 1985; Stier et al., 1981) as well as the germination and outgrowth of vegetative cells plus toxin formation (Anderson et al., 1966; Dezfulian and Bartlett, 1987; Fernandez et al., 1969). Grecz et al. (1971) monitored gas production in cans incubated for 6 months as a positive indicator for presence of C. botulinum.

The botulism risk is especially important (when the non-proteolytic strains are present) if there is temperature abuse of the product. With potentially no detectable spoilage due to suppression of spoilage organisms by irradiation and thus extended shelf life, toxin may be produced by the C. botulinum prior to detectable spoilage by the consumer. Conner et al. (1980) have reviewed this potential hazard in shelf life extended refrigerated foods. The non-proteolytic C. botulinum type #, which generally contaminates fish, can growth above 3 °C. Vacuum and low oxygen MAP packaged fish are especially a concern as toxin was produced in fish inoculated with psychotrophic C. botulinum type E strain

which was stored at refrigerated (~3°C) as well as at higher abuse temperatures (Baker and Genigeorgis, 1990; Lilly and Kautter, 1990; Pelroy et al., 1985; Stier et al., 1981). The C. botulinum strains found in meat (proteolytic type A and B) require a temperature of at least 10°C for the germination of spores, and the media or food substrate as well as the interaction of all the other factors have a direct effect on the minimum temperature required for germination (Hauschild, 1989; Sperber, 1982). This is of importance as Van Garde and Woodburn (1987) found that the normal temperature maintained in 20% of home refrigerators sometimes exceeded 10°C. Conner et al. (1989) found that at pH levels below 4.8, C. botulinum is unable to germinate at any temperature in food products, except in some very specific lab media. Competition from other microflora such as lactic acid bacteria has been shown to inhibit the growth of C. botulinum due to reduction in pH and production of antibiotic compounds (Hauschild, 1989; Gombas, 1989). Sugiyama and Rutledge (1978) found that they could inhibit the growth and toxin production by inoculated C. botulinum in mushrooms, by increasing the oxygen content in the package headspace. Lund et al. (1984) found the addition of oxygen into media filled vials reduced the growth of C. botulinum type E. They noted that due to the interaction between oxygen concentration and Eh in a food or media, both factors need to be taken into consideration regarding inhibition of C. botulinum.

The radiation D_{10}-value of C. botulinum is approximately 10 times greater than that of normal spoilage and pathogenic vegetative bacteria. Factors that

affect D_{10}-values (Banwart, 1979; Singh, 1987) of vegetative cells, such as food type, temperature, percent oxygen, and a_w at the time of irradiation, affect destruction of spores as well (Maxcy, 1983). Grecz et al. (1971) found the D_{10}-value to be 3.85 kGy in canned, cooked ground beef, irradiated at 0 °C, for type 33A spores.

3.2.2 C. sporogenes

Non pathogenic C. sporogenes has been used as an indicator organism for C. botulinum in the canning industry since the thermal D_{10}-value of sporogenes is greater than that of botulinum (Russel, 1982). Russel (1982) reviewed the work of other researchers and found the radiation D_{10}-value of C. sporogenes spores is also greater than that of C. botulinum spores in similar media/food. Other researchers have also used C. sporogenes as an indicator organism for C. botulinum while investigating chemical methods (curing agents or salts) of inhibition (DeFreitas et al., 1988a; DeFreitas et al., 1988b; Madril and Sofos, 1986; Molins et al., 1986; Pivnick et al., 1970; Robach, 1979; Sofos, 1986a; Sofos, 1986b; Whiting et al., 1984; Whiting et al., 1985; Whiting et al., 1986; Vareltzis et al., 1984). Shamsuzzaman et al. (1990) have determined the D_{10}-value of C. sporogenes in buffer, growth media and foods increasing in that order.

After reviewing other work Robach (1979), Savani and Harris (1978) and Whiting et al. (1986) expressed confidence in using C. sporogenes PA 3679 as an indicator organism for proteolytic strains of C. botulinum, C. botulinum type A,

and C. botulinum in general, respectively.

The inhibitory effects of oxygen on the germination of C. botulinum have also been identified with C. sporogenes (Sarathchandra et al., 1977). In earlier work Sarathchandra et al. (1974) determined that the three growth stages of C. sporogenes are sensitive to oxygen with cell division being the most sensitive stage followed by outgrowth and lastly germination. Carbon dioxide enhanced the germination of C. sporogenes especially in media with pH below 7.0 (Enfors and Molin, 1978). Very recently Lambert et al. (1991) have also found that certain levels of CO_2 trigger germination of C. botulinum spores. Processes that render a product free from germination and outgrowth of inoculated C. sporogenes spores are also considered safe from concerns of botulism. C. sporogenes was selected as the indicator organism for this research work due to it's applicability and added safety compared to C. botulinum.

4. MATERIALS and METHODS

4.1 MATERIALS

4.1.1 Hamburger

Bulk, fresh lean ground beef (<17% fat, Health and Welfare Canada, 1988) was purchased from a large retail outlet, (Canada Safeway, Winnipeg, Manitoba). The ground beef was prepared from fresh trim and fresh or previously frozen fat, ground in a commercial Hobart meat grinder. The ground beef was placed in a cooler and packed with ice for the 100 km trip to the Whiteshell Research Laboratories, near Pinawa, Manitoba.

4.1.2 Patty Forming Equipment

A patty forming equipment was constructed which consisted of:

(i) Patty Forming Tube: Lucite cylinder (5 cm inside diameter, 20 cm length),

(ii) Plunger: with 5 cm diameter teflon head,

(iii) Separator disks: 5 cm diameter polycarbonate circles.

4.1.3 Packaging Equipment

A Bizerba model 2002 packaging machine was used to package patties for

vacuum packaging and vacuum packaging with nitrogen gas backflush. A 99.99% nitrogen gas cylinder (Union Carbide Gas) supplied the backflush gas via a pressure regulating valve. The backflush gas was supplied at approximately 33 kPa.

4.1.4 Packaging Material

4.1.4.1 Inner Package

Cryovac 60 gauge, D-955 film, a multilayer crosslinked polyolefin film was used to make the inner bags. With an oxygen transmission rate of 8,900 $cm^3/(m^2.24h)$ and water vapour transmission rate is 0.095 $2/(m^2.24h)$.

The inner bags were constructed by trimming 30 cm centrefold (C.F.) D-955 film to yield a 15 cm (C.F.) film. Heat sealing the two 15 inch edges of the film with a single seam using a bar sealer with a heat setting of 6 gave the required 10 x 15 cm sized bags.

4.1.4.2 Outer Package

Surevak XPAE 2050 (Winpak, Winnipeg), 22 X 33 cm bags, were cut to make 15 X 20 cm. bags. The oxygen transmission rate of these bags provided by the supplier is 8-10 (cc/sq.M./24h) and the water vapour transmission rate is 4.96 ($g/M^2./24h$ @ 37.8° C, 90% R.H.).

4.2 METHODS

4.2.1 Sterilization/Sanitization

Sterilization of all equipment and media was attained by autoclaving for 15 min at 121° C (15 psi, 100 kPa) unless stated otherwise. Sanitization of equipment which could not be heat sterilized was attained by soaking in a 70 % ethanol solution.

4.2.2 Sample Preparation

Ground beef patties were prepared in a walk-in cooler (4° C) using the sanitized patty forming device described in section 4.1.2. Ten (20 ± 0.5g) portions of lean ground beef were weighed on a Mettler Model PE 3600 digital balance, and placed in the forming tube with a sterile fork. Each hamburger portion was separated by a separator disc, prior to being compressed into a consistent patty shape by the insertion of the sanitized forming plunger and applying adequate force to create consistent patties. This operation was repeated until 10 patties were stacked in the forming tube, then all 10 patties were expelled with the plunger and packaged prior to forming subsequent patties.

4.2.3 Packaging

All packaging operations were carried out at 4° C. Individual patties were placed in 10 X 15 cm inner gas permeable bags using sterile tweezers. The open end of the patty containing bag was placed over the gas flush nozzle on the

packaging machine. The settings on the Bizerba vacuum packaging machine were; vacuum: 4.0 (resulting in approximately 0.95 mbar ~1 kPa), gas flush: 8.0, and heat seal: 4.5, with a nitrogen flush line pressure at about 5 psi, 33 kPa. All ten patties were packaged in the inner D-955 bags prior to repackaging each of these (the patty/D-955 bag assemblies) in a 15 X 20 cm XPAE 2050 outer barrier bag.

The outer bags were also vacuum packaged with a nitrogen back flush as above except that the vacuum settings were 2.0 (resulting in approximately 0.90 mbar <1 kPa). This lower setting was required to insure that the inner bag assembly did not rupture. Once the lot of 10 patties was dual packaged, a new lot of patties was formed and packaged.

Packaged patties were stored in the 4° C walk-in cooler until patties were randomly selected for the various treatments (usually < 1 day of preparation).

4.2.4 Sample Irradiation

All irradiations were carried out using either electron beam or CO 60 gamma source, at Whitshell Research Laboratories, AECL Pinawa, Manitoba.

4.2.4.1 Electron Beam Irradiation Experiments

Most of the irradiations were carried out using the AECL I-10/1, 10 Mev electron accelerator. Dual packaged hamburger patties placed on top of a bag of crushed ice in the bottom of 61 X 81 X 13 cm. aluminum trays. The patties were

aligned in two rows, without patty overlap. Each row was no greater than 12 cm from the longitudinal centre line to ensure even dose application (Fig. 1). The patties were covered with an 8 mil polyethylene sheet which was taped to the trays to maintain the temperature and ensure that the patties did not move during the approximately 15 min required to transport to and from the point of irradiation on the I-10/1 conveyor system. The time of irradiation under the beam was approximately 17 s. at a nominal dose rate of 150 Gy/s. Once irradiations were completed, samples were returned to the walk-in cooler.

4.2.4.2 Gammacell 220 Irradiation experiments

Samples irradiated in the gammacell 220 (dose rate 180 Gy/min) were placed in a 2 L beaker containing crushed ice to maintain sample temperature at approximately 0° C.

In experiments for determining the D_{10} values (90 % reduction) of C. sporogenes spores, the spores suspended in phosphate buffer and irradiated in screw top test tubes held vertically in the beaker by an aluminum tube cradle (Fig. 2).

Packaged hamburger patties irradiated in the gammacell were placed concentrically (6 patties per beaker) along the outer wall of the beaker and the samples were held in place by an inner beaker containing crushed ice. Patties were returned to the walk-in cooler immediately after irradiation.

4.2.5 Dosimetry

Actual dose received by the samples was determined by using Radiochromic Dosimeters (RCD). GAF and FWT dosimeters were used when samples were exposed to 0-3 kGy and 3-200 kGy of ionizing radiation, respectively. Doses absorbed by the RCDs were measured by the AECL Dosimetry Section personnel.

4.2.5.1 Electron Beam Irradiation Experiments

Operation of the I-10/1 Electron Beam Accelerator was performed by personnel in the Dosimetry section. Five dosimeters were taped to the 8 mil polyethylene sheet covering the patties at locations indicated in Fig. 1.

4.2.5.2 Gammacell 220 Irradiation Experiments

Control of the dose applied to the samples irradiated in the gammacell was regulated by the time for which the sample chamber was exposed to the Cobalt-60 source. Actual "Corrected" irradiation time required to obtain a desired absorbed dose is calculated by:

$$\text{Corrected time (min)} = \frac{\text{Time (min)}}{\text{Isodose Value}}$$

where: Isodose Value, (location correction factor) is determined from the Isodose Distribution Chart in the Gammacell 220 Standard Operating Procedures Manual.

Figure 1. Aluminum Sample Tray

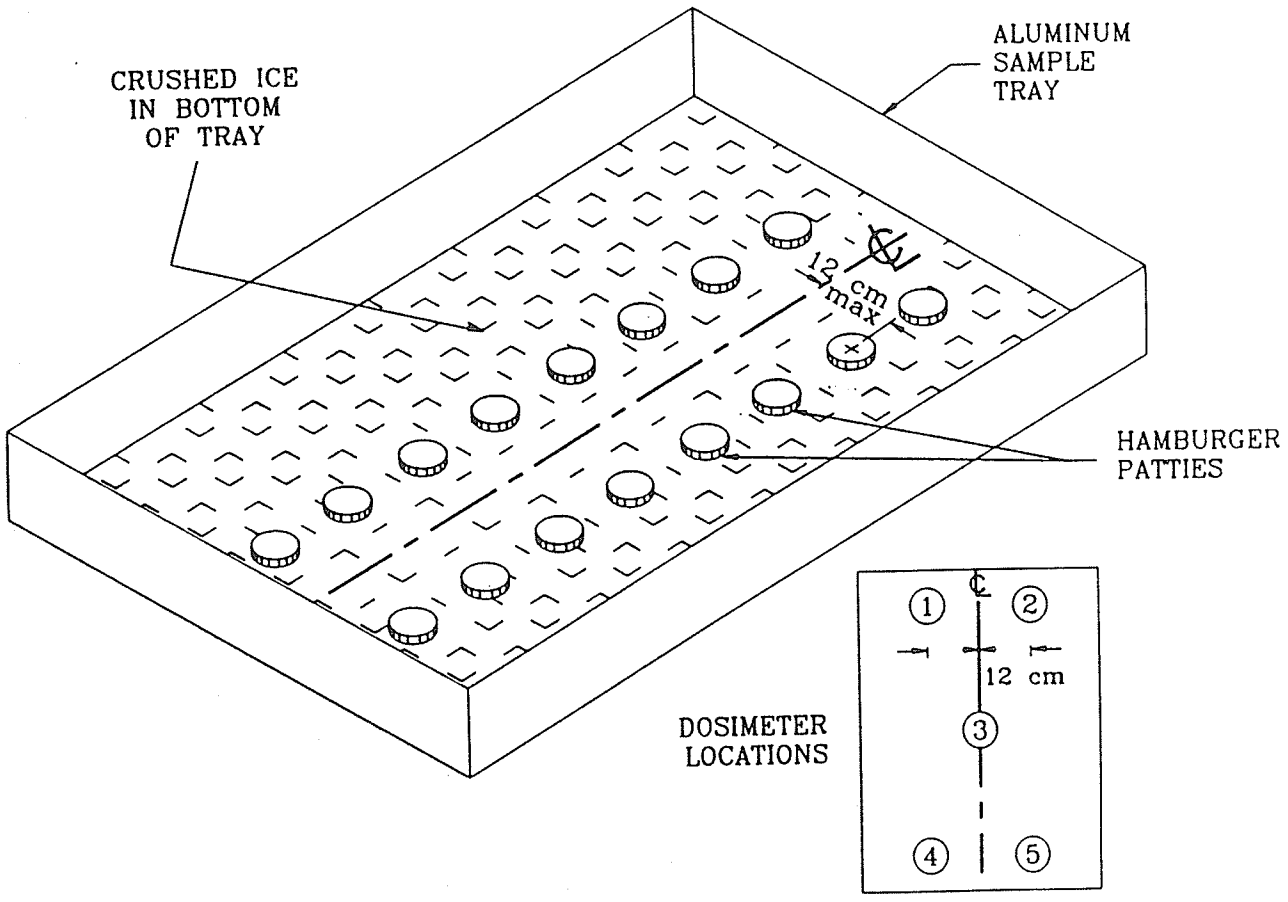
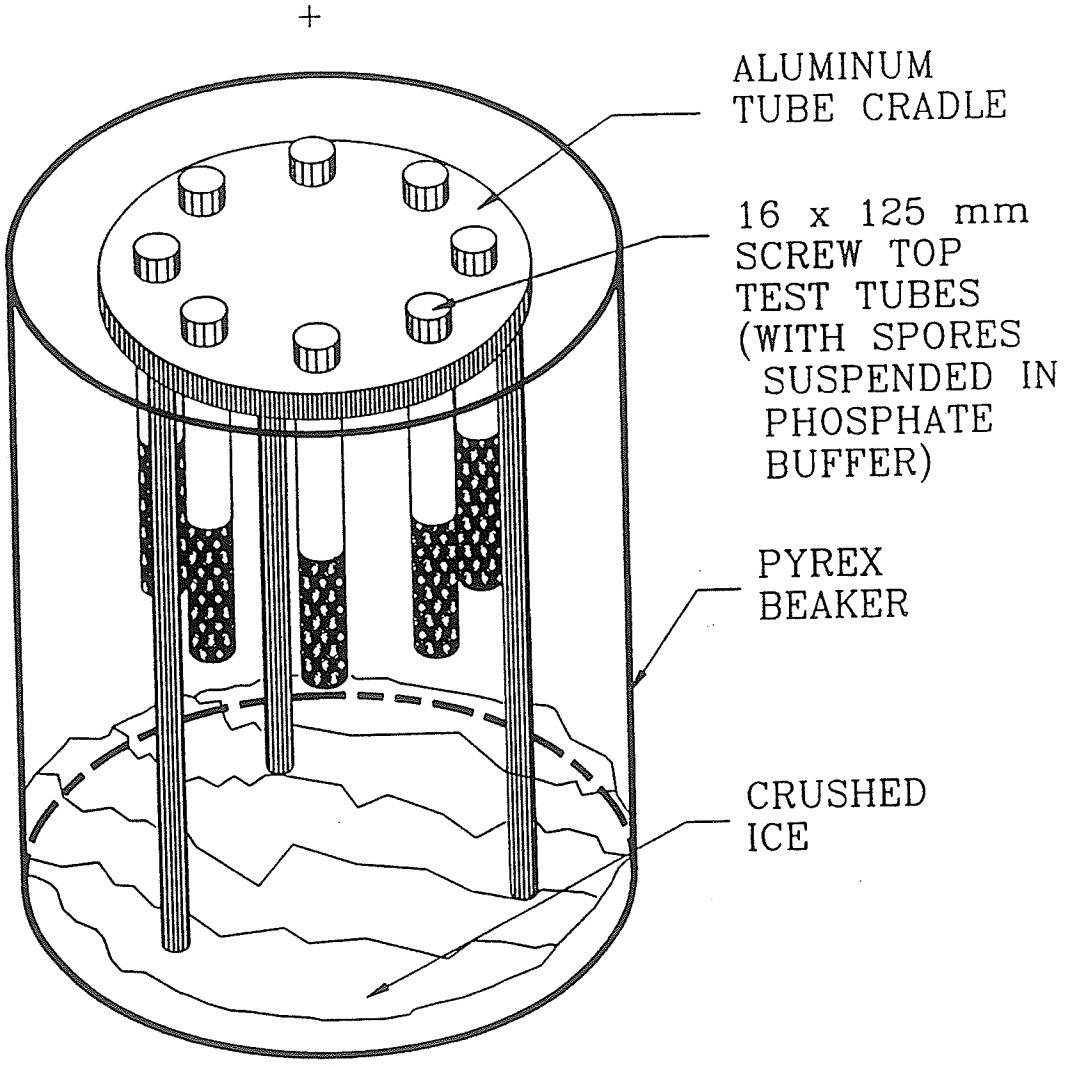


Figure 2. Aluminum Tube Cradle



Time (min), the calculated irradiation time is given by:

$$\text{Time(min)} = \frac{\text{(RD-DO) Gy}}{\text{DR Gy/min}}$$

Time = Required time to irradiate sample.

Rd = Required absorbed dose in Gy.

Dr = daily dose rate from tables (See Gammacell 220 Op. Manual)

To confirm the dose applied to:

A) Spore suspension in tubes. Two RCD dosimeters, packaged in water tight foil packages were taped to the side of each tube. One at the bottom of the tube and the other at the top surface of the spore suspension.

B) Patties. Three dosimeters were taped to the surface of randomly selected packages.

4.2.6 Storage of Samples

Patties were stored with either; the inner gas permeable bag and outer gas barrier bag intact (dual package), or with the outer gas barrier bag removed after irradiation leaving the patty and only the gas permeable bag surrounding it (single package).

All samples were returned to the walk-in cooler immediately after irradiation, and were placed on trays according to the assigned storage conditions. Samples that were assigned single package storage condition had

their outer gas barrier XPAE 2050 bag removed at this time.

Samples were stored in the dark on stackable plastic mesh trays. Packaged samples were evenly disbursed so that air was equally accessible to the top and bottom of each package. Paper towels were placed over each tray containing samples so any unintentional effect of ultraviolet radiation from lights would be eliminated. Trays containing patties with similar abuse temperature storage conditions were transferred to the appropriate locations at this time.

4.2.6.1 Storage Temperature

Samples stored at temperatures less than 20° C were placed in walk-in coolers maintained at the indicated temperatures. Samples stored at ~ 20° C were kept in an air conditioned room.

4.2.6.2 Storage Oxygen Content

Oxygen content surrounding packages during storage was ambient (21% oxygen) unless otherwise stated. When oxygen content was required to be maintained at less than ambient, samples were placed in an Aldrich model Z10,608-9 520 L. atmosbag. The oxygen content in the atmosbag was maintained at the desired level by a Oxyreducer model 311. The Oxyreducer controlled the oxygen content in the sealed atmosbag containing the sample trays by monitoring the oxygen content and regulating the flow of 99.9 % nitrogen through a 7.5 cm diameter tube into the atmosbag to maintain the desired

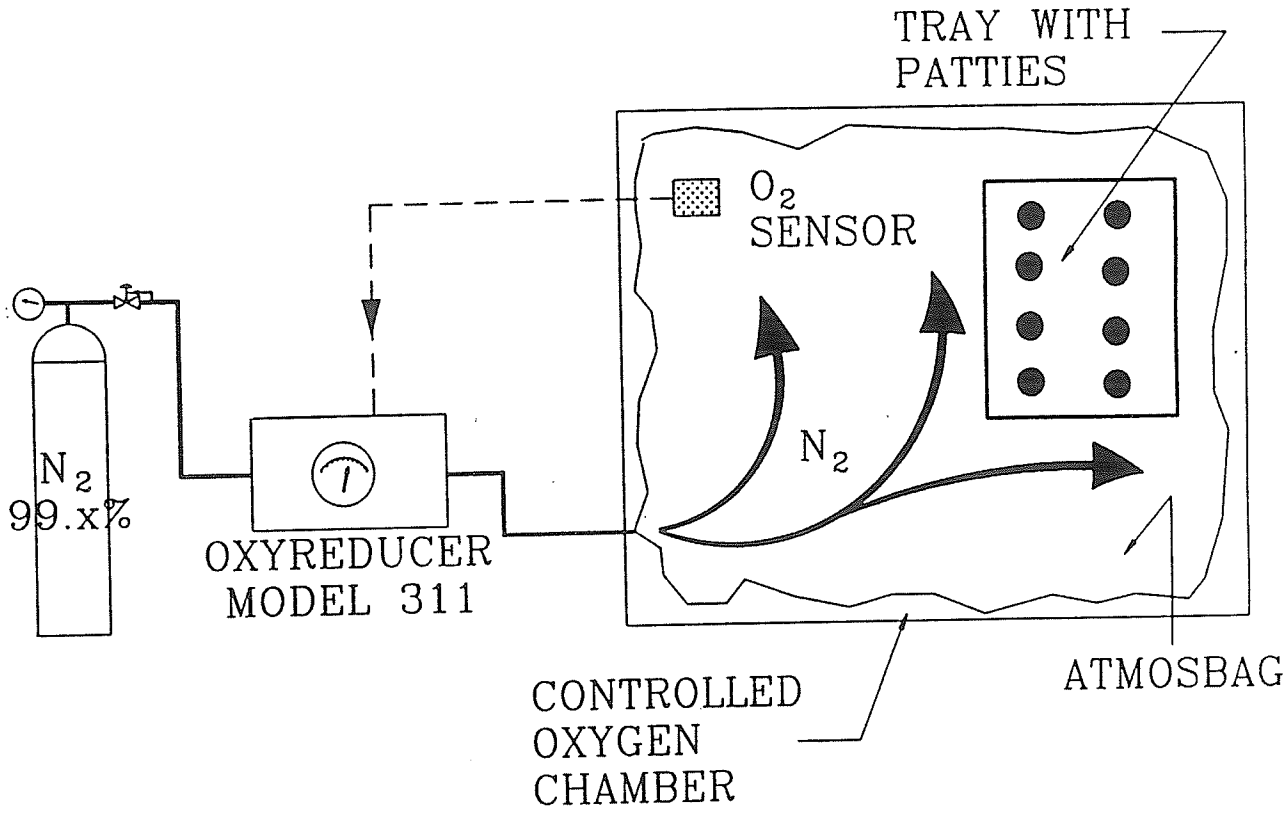
oxygen content (Fig. 3).

4.2.7 Preparation of Hamburger For Analysis

Samples selected to be monitored on any given day were removed from their storage environment and transported on ice to the microbiology lab. The packaging material was cut at one end with sanitized scissors. Aseptic technique was used in a laminar flow sterile station for the following procedures. Each patty was transferred into a Seward #400 sterile stomacher bag. Visual colour and odour were recorded at this time. Sterilized 0.1 % peptone (at 7° C) was added to the hanburger patty to give ten fold dilution (1:10) and the mixture was stomached for 1 min in a Lab-Blender model 400 Stomacher. The particulate matter was allowed to settle for approximately 1 min., before a 10 mL aliquot was taken using a 10 mL sterile pipette and stored in a sterile 16 X 125 mm screw top tube (kept on ice).

The pH of the remaining 1:10 diluted stomached patty mixture was measured at this time using a Fisher Scientific model 805 MP pH meter and Fisher model 13-639-252 pencil-thin gel filled combination electrode. The stomached patty mixture was disposed of this stage.

Figure 3. Controlled Atmosphere Storage



4.2.8 Incorporation of Ascorbic Acid

Crystalline L-ascorbic acid (0.1% by weight) was incorporated in to the ground beef by using the regrinding method of Shivas et al. (1984). The method was modified to regrinding 5 times instead of only twice using a sterile meat grinder located in the walk-in cooler.

4.2.9 Clostridium sporogenes Growth

4.2.9.1 Growth Method

The propagation and recovery method of Brown et al.(1957) as modified by Shamsuzzaman (1988) was used to produce a stock spore suspension of C. sporogenes ATCC 7955.

A single C. sporogenes colony (originally purchased from American Type Culture Collection) isolated from a enumeration plate provided by Dr. K. Shamsuzzaman was used as an inoculation source. Two tubes (15 X 150 mm) containing 15 mL of freshly sterilized and cooled trypticase thioglycolate broth (TTB) were inoculated with C. sporogene spores and incubated anaerobically at 35° C for 24 hours. This 48 hour culture was used to inoculate a freshly sterilized, cooled 135 mL quantity of TTB (in a 250 mL beaker) and was incubated anaerobically at 35° C for 48 hours. The resulting 150 mL culture was used to inoculate a 1.3 L of freshly sterilized and cooled TTB (in a 2.8 L Fernbach flask), followed by 7 days of anaerobic incubation at 35° C. (Shamsuzzaman, personal communication).

There were a total of ~ 205 mL of $1.4x10^7$ spores/mL of the combined harvested cultures were collected from two separate growth periods (GP), (GP-1: Sept./89 and GP-2: Oct./89). Each GP in turn was comprised of two batches each as two separate growth flasks were utilized with each GP of C. sporogenes inoculum growth, resulting in 4 distinct batches (Table 1).

4.2.9.2 Harvest Method

The spores were harvested by the centrifugation and washing procedure of Shamsuzzaman (1988). The contents of the 1500 mL C. sporogenes culture were equally divided among six, 250 mL sterile centrifuge bottles which were centrifuged at 9000 X g for 15 min at a temperature of 4° C. The resulting supernatant were decanted and the dark grey pellets combined into one centrifuge bottle with a sterile stainless steel spoon while stored on ice. Approximately 100 mL of 0.067 molidum phosphate buffer (adjusted to pH 7.0 with 1 N HCl) were added to the pellet to wash the spores. The pellet suspension was stirred for 30 min by a sterile stir bar while on ice followed by centrifugation at 9000 X g for 15 min at 4° C. The supernatant was decanted off and the wash procedure was repeated 3 times. The washed spores pellet was finally suspended in 50 mL of 0.067 M phosphate buffer.

4.2.9.3 Storage of Spores

The C. sporogenes suspension was stored in the 250 mL centrifuge bottles

at ~ 5° C.

4.2.9.4 Evaluation of C. sporogenes Suspension

The total number of C. sporogenes (vegetative bacteria and spores) was determined by serial dilution and plate count enumeration of the suspension on Pflug's Medium anaerobically incubated for 48 hours at 35° C. The number of C. sporogenes spores were determined by heat treating the 10^1 dilution tube in a water bath at 80° C for 15 min. as outlined by Madril and Sofos, (1986) to destroy all vegetative bacteria. Subsequent dilutions were made from the heat treated dilution tubes.

$$\% \text{ Spores} = \frac{\text{Number of Spores}}{\text{Total Number of Vegetative Bacteria and Spores}}$$

Table 1. C. sporogenes Spore Recovery from Different Growth Batches

(In 0.067 M Phosphate Buffer)

Growth Period	Batch #	Volume (mL)	Clostridium Total/mL[1]	Sporogenes Spores/mL	(%) Spores
1 Sept/89	1	40	3.7×10^7	2.3×10^7	62
1 Sept/89	2	55	2.7×10^7	1.8×10^7	62
1 Oct/89	3	55	1.8×10^7	1.4×10^7	77
2 Oct/89	4	55	8.6×10^6	6.4×10^6	74
Combined Total		205	NA[2]	1.4×10^7	NA

1. Total number of vegetative bacteria and spores.
2. NA: Not available.

4.2.9.5 Preparation of Spore Inoculum

The spore preparation was heat treated to destroy all vegetative bacteria and insure that the inoculum consisted of only spores. The centrifuge bottles containing the C. sporogenes suspensions and a bottle containing 0.067 M phosphate buffer blank were placed in a water bath at 80° C. The temperature of the blank was monitored by a Cole Palmer model 8110-20 Thermistor thermometer and the samples were placed in ice water after being maintained at 80° C for 15 min. There were ~ 205 mL of $1.4x10^7$ spores /mL of the combined inoculum (Table 1).

Prior to each inoculation pack experiment an aliquot of the stock spore suspension was again heat treated in a 16 X 125 mm screw top tube with the appropriate blank to insure adequate heat treatment.

4.2.10 D_{10} Values of Spores

Radiation D_{10} of the spore suspension is the dose (kGy) to reduce the population by 90 % (1 Log10). The D_{10} was determined by applying incremental irradiation doses (Gammacell 200) to an initial population of spores and plotting the number of survivors. A best fit curve was calculated for these data, and the dose (kGy) to reduce a given population by 90 % (1 Log10) was determined from the best fit line. The flow diagram outlining this experimental procedure is shown in Fig. 4.

Figure 4. Flow Diagram: D_{10} Value Determination of Spores

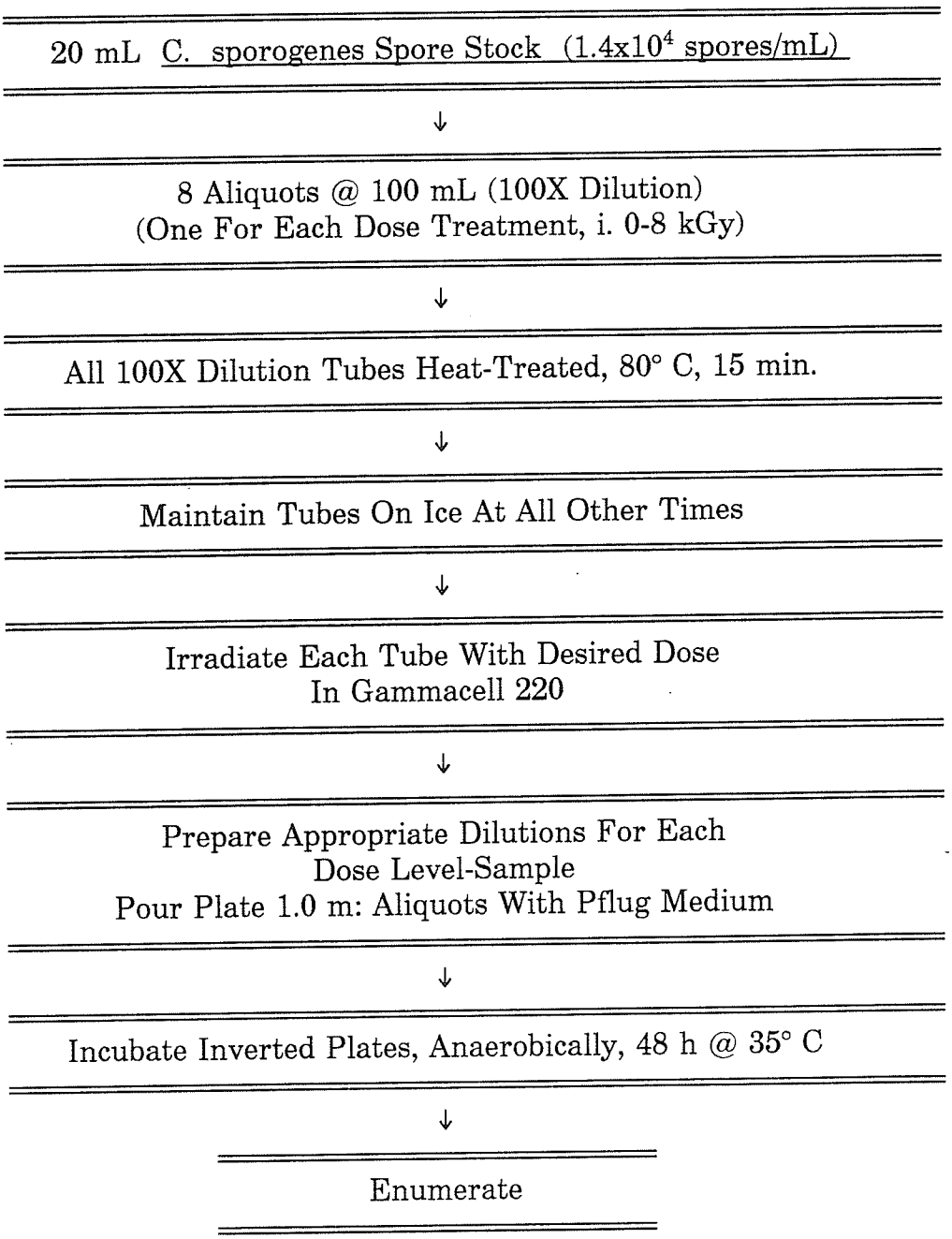


Figure 5. Flow Diagram: Inoculation Pack Study Sterilied Ground Beef

Prepare and Package Patties; in 4° Walk-in Cooler
1) Place Patty in Inner Bag (Do Not Vacuum Package)
or
2) Vacuum Package With Nitrogen Backflush, Open Inner Package - Patty Assembly in Barrier Bag

↓

Sterilize With 30 kGy, on Ice (Using I-10/1 e-Beam)

↓

In Sterile Laminar Flow Hood, Cut Open Outer Barrier Bag;
Remove Inner Bag Containing Patty,
Surface Inoculate With C. sporogenes Spores

↓

In 4° C Walk-in Cooler
1) Vacuum Package With Nitrogen Backflush
Inner Package Containing Inoculated Patty
or
2) Vacuum Package With Nitrogen Backflush
Inner Package-Patty Assembly in Outer Barrier Bag

↓

Using I-10/1 or Gammacell 220, 2.5 kGy Sanitize
Inoculated Dual Package Patties (On Ice)

↓

In 4° C Walk-In Cooler
Remove Outer Barrier Bag From Samples
To be Stored With Only Single Packaging

↓

Store Patties On Plastic Mesh Trays At Appropriate;
Temperature, Packaging Condition, Oxygen Concentration

4.2.11 C. sporogenes Inoculation of Ground Beef

All patties were inoculated to achieve approximately $5x10^4$ spores/g prior to any subsequent radiation treatment. A concentration of approximately $1x10^4$ spores/g remained after subsequent radiation treatments of 2.5 kGy.

4.2.11.1 Surface Inoculation (Sterilized Patty Experiments)

Sterilized (30 kGy) dual packaged patties were surface inoculated by evenly distributing 71.4 ($1.4x10^4$ spores/mL) of heat treated spore stock (stored on ice) on the patty surface with a Eppendorf Adjustable Pipette in a sterile laminar flow hood. The patties were immediately returned to the walk-in cooler where they were repackaged as in section 4.2.3. The flow diagram outlining this experimental procedure is depicted in Fig. 5.

4.2.11.2 Homogeneous Inoculation (Non Sterilized Patty Experiments

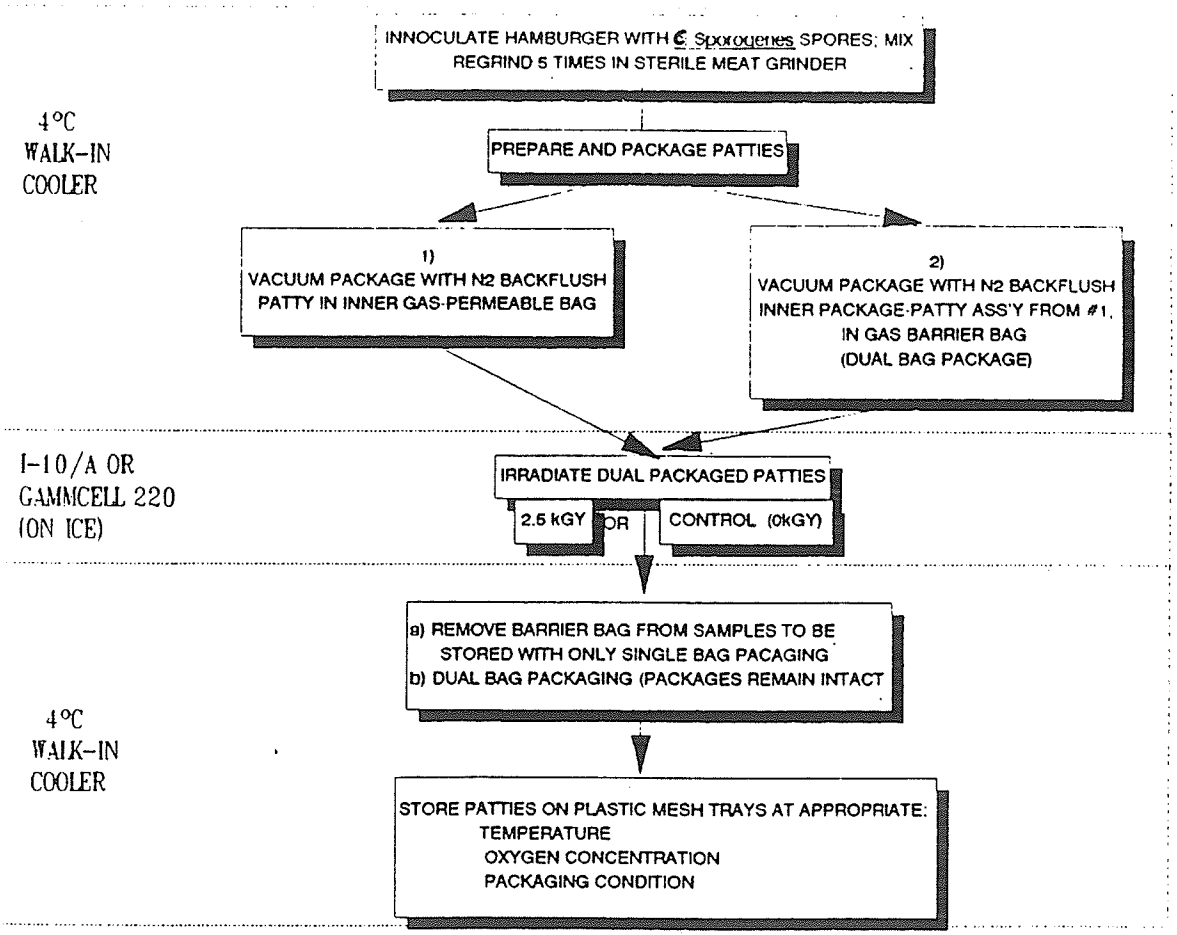
Homogeneous inoculation of ground beef patties was achieved by applying an appropriate volume of spore suspension on ground beef and then regrinding the meat 5 times through a sterile meat grinder as outlined by Firstenberg-Eden et al. (1980). This was done in the walk-in cooler at ~ 2° C. The effectiveness of incorporating spores homogeneously by this method was verified by the results from the following experiment. Fresh ground beef kept on ice was sterilized with a 25 kGy (electron beam) dose. A 550 g portion of the sterilized ground beef was inoculated with 0.40 mL of a $1.4x10^7$/mL clostridial spore suspension. This

resulted in a calculated spore concentration of $1.0x10^4$/g in the ground beef. The inoculated ground beef was reground 5 times in a sterile meat grinder, to insure homogenous distribution of the spore inoculum. The regrinding process took place in a walk-in cooler at 2° C. Pflug's medium was used to evaluate the number of clostridial spores and the total clostridia. Non heat-treated stomached samples (Pflug-NH) and heat-treated stomached samples (Pflug-H) were used to monitor the total clostridia and the clostridial spores, respectively. Four randomly selected patties were evaluated to determine the homogeneity of the inoculum. The initial number of spores found in four random samples were $1.5x10^4$/g (± $0.12x10^4$/g) (Table 2). This minimal variability justified using the regrinding procedure (Shivas et al., 1984) to homogeneously distribute the inoculum in the ground beef. The flow diagram for this procedure is shown in Fig. 6.

Table 2. Homogeneity of C. sporogenes Inoculum After Regrinding Meat

Sample	Dilution	CFU#1	CFU#2	CFU-Avg	Concentration Spores/g of hamburger
1	10^2	156	173	164.5	$1.6x10^4$
2	10^2	165	120	142.5	$1.4x10^4$
3	10^2	151	130	140.5	$1.4x10^4$
4	10^2	167	166	166.5	$1.7x10^4$
				153.5 ± 12.0	$1.5x10^4$

Figure 6. Flow Diagram: Fresh Ground Beef Study (Inoculum Mixed in Meat)



4.2.12 Microbiological Assays

Standard aseptic techniques and procedures as outlined in (APHA, 1984) were utilized as follows. Stomached patties and liquid spore suspensions were serially diluted with 0.1 % peptone. The 1:10 dilutions were prepared by aseptically adding 1.0 mL into 9.0 mL of diluent and 1:100 dilutions by adding 0.1 mL into 9.9 mL of the diluent. Mixing on a vortex mixer for 10 s. insured a homogeneous mixture. Dilution tubes were kept on ice and 0.5 mL aliquots from desired dilutions, were pour plated in duplicate unless otherwise stated.

All media were prepared with distilled water and recommended quantities of dehydrated media, as per label directions. Freshly sterilized media were tempered to 45° C in a water bath prior to pour plating with approximately 15 mL of medium per 100 X 15 mm Baxter petri dishes. Inocula and media were mixed by stirring plates on a plate stirrer before being allowed to solidify. Plates were incubated in an inverted position with the media used, and the incubation conditions are listed in Table 3.

Where required, anaerobic conditions were created by a BBL Gaspak 100, or 150 Anaerobic System. The H_2 and CO_2 generator envelopes along with the palladium catalyst created the anaerobic environment as confirmed by disposable anaerobic indicators.

Micro-aerophilic conditions were created for lactobacilli in MRS plates using an overlay method recommended by Sharpe (1960) when availability of anaerobic jars dictate as stated in Oxoid (1972). The inoculated and solidified

MRS plate was overlayed with approximately 15 mL of MRS.

Colony forming units were determined on a Colony Counter and plates with between 30-300 colonies were recorded according to the standard methods (APHA, 1984).

Table 3. Microbial Assays, Media and Incubation Conditions

Assay	Micro. Monitored	Media	Incubation Condition Temp/Time/ Atmosphere	Ref.
APC	Aerobic Meso	PCA	35°C/48 h/aerobic	HPB
PSY	Psychrotrophs	PCA	10°C/ 7 d/aerobic	HPB
Y/M	Yeast & Mould	PDA+acid	20°C/ 5 d/aerobic	APHA
LAB	Lactics	MRS/olay	20°C/ 5 d/overlay	Oxoid
LAB	Lactics	MRS/olay	35°C/48 h/overlay	Oxoid
TAC	Total Anaerobes	SC+2	35°C/48 h/anaerobic	Sec 5.1
Tot C	Total Clostridia	SC+2	35°C/48 h/anaerobic	Sec 5.1
C spor	C. spores	SC+2	35°C/48 h/anaerobic	Sec 5.1

4.2.12.1 Aerobic Plate Count

The Aerobic Plate Count (APC) was determined according to MFA-18 (HPB, 1982), with modifications as above. At low (10^1, 10^2) dilutions, differentiation between the food particles and the colonies on the incubated Plate Count Agar (PCA) plates was aided by flooding the plates with 2 mL of 0.1% 2,3,5, triphenyltetrazolium chloride, pouring off excess solution, and counting the colonies which reduced the indicator thus yielding red colonies after 3 hours of inverted incubation at room temperature.

4.2.12.2 Psychrotrophs

The psychrotrophic colony count (PSY) was determined according to HPB, (1982).

4.2.12.3 Yeast and Moulds

The number of Yeast and Moulds (Y/M) was determined according to APHA (1984) as follows. Potato Dextrose Agar (PDA) was prepared as per label instruction including the acidification of the tempered media to a pH of 3.5 by the addition of 1.6 mL of a filter sterilized 10% solution of tartaric acid to each 100 mL of agar.

4.2.12.4 Lactic Acid Bacteria (LAB)

Lactobacillus MRS broth plus 1.5% agar were combined to produce MRS Agar, a method utilized by Lee et al. (1984). The MRS agar can be used to cultivate the whole group of lactic acid bacteria (Lab) as described by Baird et al. (1987). Two of their recommended incubation time/temperatures were modified due to limitations of incubator availability. The recommended 37° C 48 hours and the 25° C 3 days confirms to monitor mesophyllic and mesophyllic-psychrotrophic LAB from meat, respectively were modified to 35° C 48 hours and 20° C 5 days.

Characteristic colony size is given by Baird et al. (1987) as:

Lactobacilli:	0.5-2.5 mm diameter
other lactic acid bacteria:	0.5-2.0 mm diameter
enterococci:	0.5-1.0 mm diameter

The colony appearance was usually small greyish white, flat or raised, smooth, rough or intermediate.

4.2.12.5 Anaerobic Plate Count (AnPC)

Anaerobic Plate Count (AnPC) is defined as the number of white colonies appearing on PFlug, SC+3 or SC+2 agar when anaerobically incubated.

Various methods have been utilized to monitor "AnPC" in ground beef for example, Molins et al. (1986) utilized Trypticase Soya Agar and Whiting et al. (1984) used Botulinum Assay Medium (BAM), incubated anaerobically at 35° C 48 hours and 30° C 48 hours respectively.

4.2.12.6 Total Clostridia (Vegetative and Spores)

The SC+2 medium developed during this work (see section 5.1) was used to monitor total Clostridia. The SC+2 is prepared by sterilizing 23.5 g of dehydrated SFP media plus 500 mL of distilled water. Five millilitres of each of the following filter sterilized solutions (stored for no more than 1 week at 7° C) were added to the tempered base medium.

12.5% sodium thioglycolate,

10 % sodium bicarbonate,

4 % d-cycloserine,

Appropriate dilutions were plated, incubated anaerobically at 35° C for 48 hours and the black colonies (1-5 mm diameter) counted.

4.2.12.7 Clostridial Spores

The SC+2 media listed above was also used in the enumeration of clostridial spores. To destroy all vegetative bacteria and to enumerate only spores, a heat treatment of 80° C for 15 min as described by Madril and Sofos (1986) was utilized. The heat treatment was monitored by noting the temperature in a dummy dilution tube along side the actual sample tubes. Once 80° C was reached and maintained for 15 min., the tubes were immediately put on ice. The heat treatment was applied only to the 1:10 dilution tubes prior to appropriate serial dilutions, these subsequent dilution tubes were also denoted as heat treated.

Plates were incubated anaerobically at 35° C for 48 hours and the number of the black colonies (1-5 mm diameter) that grew were recorded.

4.2.12.8 Pflug Medium

Initially Pflug's YEA medium (Pflug et al. 1979) modified as follows was used to monitor either the total clostridia or the clostridial spores alone, based

on no heat-treatment or heat-treatment, respectively, as discussed above.

Yeast extract:	5.0 g
Soluble starch:	0.5 g
K_2HPO_4 potassium phosphate:	1.0 g
Agar:	7.5 g
Distilled water:	500 mL

These components were combined and sterilized. The following filter sterilized solutions (5 mL each) were added aseptically to the 500 mL of tempered sterilized medium:

12.5% sodium thioglycolate

50% dextrose

10% sodium bicarbonate

4.2.13 Microbial Spoilage Level

The microbial spoilage range for ground beef was given by Jay and Shelf (1978) as $3.2x10^7$ - 1x10 8/g) APC. In this study we have used values of ~ 5x10 7/g APC as the spoilage criterion for ground beef.

4.2.14 Chemical / Physical Measurements

Colour, pH, and odour were measured or noted as described in section 4.2.7, except where the colour measurement was done, which is described below.

4.2.14.1 Colour Measurement

Objective colour measurements of patty colour were made during the refrigerated shelf life extension and ascorbic acid addition experiments. A Hunterlab model D-25L-2 Color/Difference Meter with a 5 cm sample viewing port was used. Hunter L, A, and B measurements for both sides of the patties were taken.

The unit was calibrated according to the manufacturer's instructions, as follows: The Sensor calibration was confirmed with the colour plates provided by the manufacturer. To allow for the colour measurement through the inner packaging film, as was done by Rhee et al. (1985), further calibration using the black and white colour plates covered with a single layer of D955 film (the single bag packaging material) was performed. A wood box lined with black felt was placed over the samples and the sampling port to insure that ambient light had no effect on the Hunterlab readings. Care was taken to present each packaged patty in a similar manner to the sample port. Each patty was slightly pressed between two hands to expand the diameter of the patty to insure that the entire 5 cm sample port was covered. The patty was centred over the sample port, and the patty surface was flushed with the top of the sample port.

The colour measurements were taken immediately after the samples were removed from the walk-in cooler. The outer barrier bag was removed from the dual packaged stored samples, so that all the samples were monitored through only the D955 film. The samples from the shelf life extension experiment were placed on ice immediately after the colour measurements and taken to the

microbiology lab to be prepared for microbiological evaluation (see section 4.2.7). The samples from the ascorbic acid experiment were returned to the walk-in cooler for subsequent monitoring.

4.2.14.2 Oxygen Content Within Package

Oxygen content within the sample bags was monitored with a Mocon LF-700D Oxygen Headspace Analyzer. The instrument was calibrated and operated in the sample injection mode according to the manufacturer's instructions, as follows. The needle of a gas tight syringe was inserted into the package through an adhesive rubber septum affixed to the package surface. A 5 mL gas sample was removed from the package headspace, and injected into the sample injection port of the analyzers at a rate of approximately 1 mL/s. The measured oxygen content was recorded.

Limited availability of the analyzer from the accounted for limiting the monitoring of the oxygen content to initial experiments.

5. RESULTS

5.1 Radiation Survival of Clostridium sporogenes

The data for the counts of C. sporogenes spores from all four batches suspended in 0.067 molidm-3 phosphate buffer (pH, 7.0) surviving various doses of gamma radiation are shown in Figs. 7 and 8. The non-exponential inactivation of spores generally show an initial shoulder on the curves in both figures (Figs. 7 and 8) followed by an exponential decline is typical for C. sporogenes (Shamsuzzaman et al. 1990) and also for C. botulinum types A, B, and E, (Russel,1982). The shoulder for all the four batches tested extends to ~ 3 kGy, followed by the exponential destruction of the spores. The resistance of an organism to a given treatment is usually represented by the D_{10} of the organism. The D_{10} value is the dose (kGy) required to reduce the initial spore population by 90%. The D_{10} values for the four individual batches and the combined batch (used for inoculation studies) of spores, calculated from their radiation survival data (Fig.7 and 8), are given in Table 4. Figure 9 shows the radiation survival results for the combined batch. The best fit line for the combined batch, calculated by linear regression is shown as well (Figure 9). The shoulder on the curve is not as pronounced as seen with the individual batches of spores (Fig. 7 and 8). The D_{10} value of the combined inoculum was 3.37 kGy (Table 4).

Figure 7.

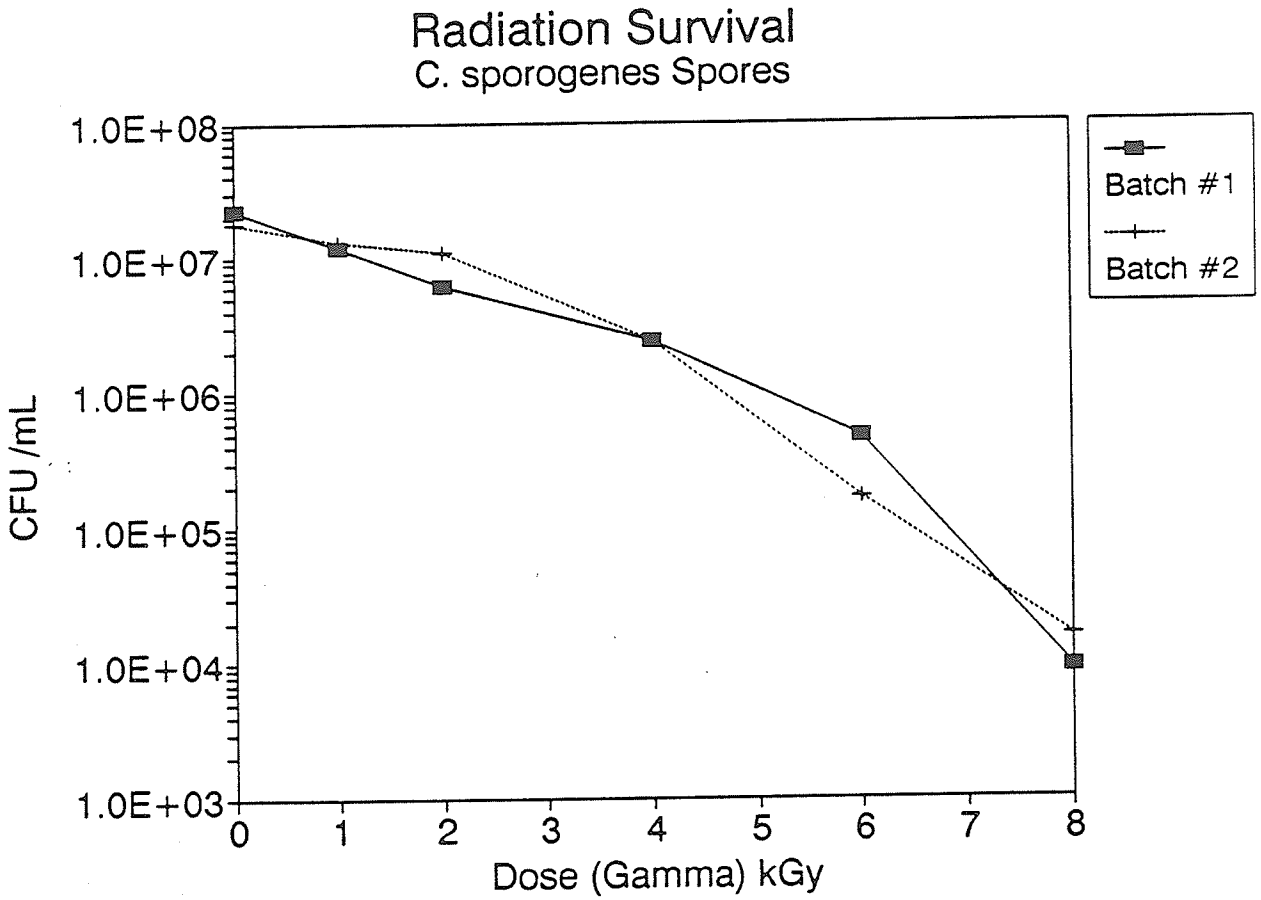

Radiation Survival
C. sporogenes Spores
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
CFU /mL
0
1
2
3
4
5
6
7
8
Dose (Gamma) kGy
Batch #1
Batch #2

Figure 8.

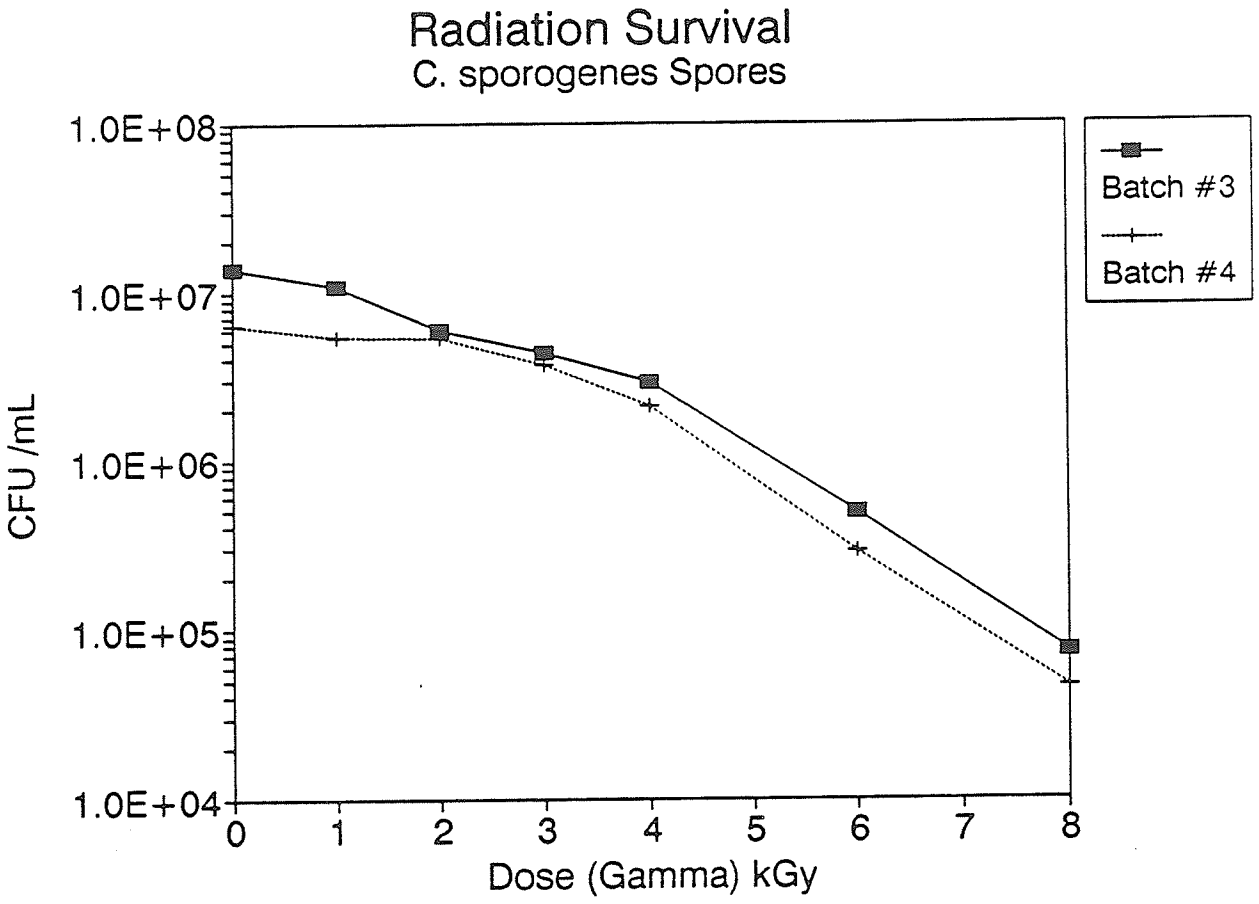

Radiation Survival
C. sporogenes Spores
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
CFU /mL
0
1
2
3
4
5
6
7
8
Dose (Gamma) kGy
Batch #3
Batch #4

Figure 9.

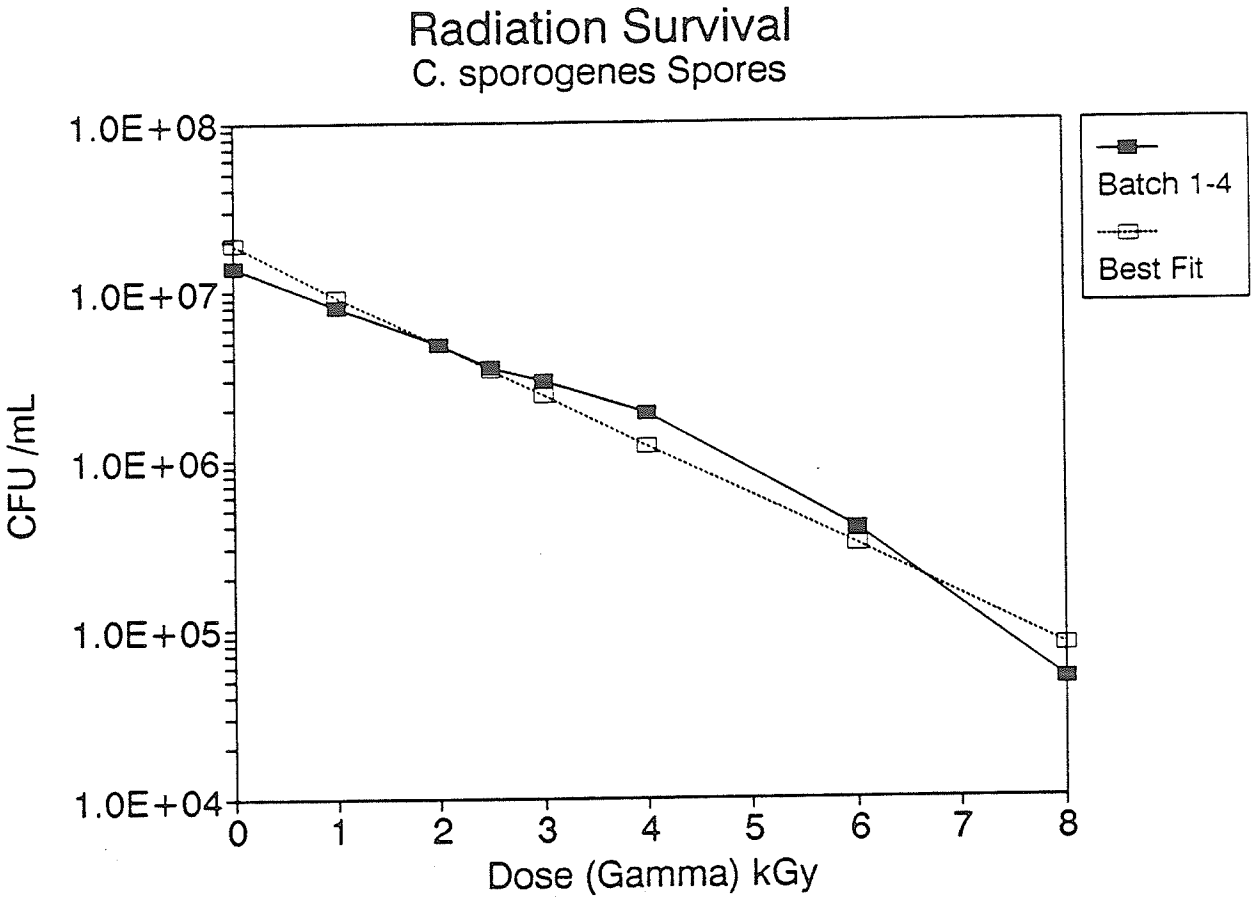

Radiation Survival
C. sporogenes Spores
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
CFU /mL
0
1
2
3
4
5
6
7
8
Dose (Gamma) kGy
Batch 1-4
Best Fit

Table 4. D_{10} of C.sporogenes Spore Inoculum (In 0.067 M Phosphate Buffer)

Growth Period	Batch #	Volume (mL)	Clostridium Sporogenes Total/mL	Clostridium Sporogenes Spores/mL	Radiation D_{10} Value
1 Sept/89	1	40	$3.7x10^7$	$2.3x10^7$	2.70
1 Sept/89	2	55	$2.7x10^7$	$1.8x10^7$	2.88
2 Oct/89	3	55	$1.8x10^7$	$1.4x10^7$	3.18
2 Oct/89	4	55	$8.6x10^6$	$6.4x10^6$	3.41
Combined Total		205	NA[2]	$1.4X10^7$	3.37

1. Total = vegetative + spores
2. NA: Not available

5.2 Method Development to Monitor Inoculated C. sporogenes in Ground Beef

During the course of inoculation pack experiments it became evident that the initial method of monitoring C. sporogenes was ineffective for counting the total clostridia (spores and vegetative). The high number of the facultative bacteria present in ground beef (unirradiated control and radurized), interfered with the enumeration of inoculated C. sporogenes from the non-heat treated extracts using the Pflug media (total clostridia) assay. This demonstrated the need to develop a selective / differential assay to monitor total C. sporogenes as the methods described in literature were not effective with the high microflora background present in ground beef. To facilitate understanding of the method

development, all the media that were used in these experiments are as follows:

5.2.1 Inoculation Concentration Gradient Experiment (Pflug Media)

An experiment was conducted to determine if fresh ground beef inoculated with varying amounts of C. sporogenes spores could be monitored accurately with Pflug media in the presence of the normal microflora. Non heat-treated stomached samples (Pflug-NH) and heat-treated stomached samples (Pflug-H) were used to monitor total clostridia and clostridial spores, respectively. C. sporogenes spore inoculation levels used were: 0, 10^2, 10^3, 10^4, 10^5, and $5x10^5$/g. The results for this experiment are shown in Fig. 10. The average APC for all the samples was ~ $7.9x10^6$/g. The total clostridia (Pflug-NH) assay on average was only 0.25 Log10 lower at ~ $4.4x10^6$/g. This demonstrated that the Pflug-NH assay was not selective enough for use in fresh ground beef. A large portion of the organisms monitored with the APC are also monitored on the anaerobically incubated Pflug-NH assay for total clostridia. The organisms monitored on the Pflug-NH assay may be considered to be AnPC. This high percentage of anaerobic and/or facultative anaerobic organisms agree with what is reported in the literature. As Madden and Moss (1987) found, the AnPC:APC ratio to be between 0.6 Log10 lower-1 Log10 higher depending on the condition of the meat and fat used to prepare the ground beef. Most of the natural

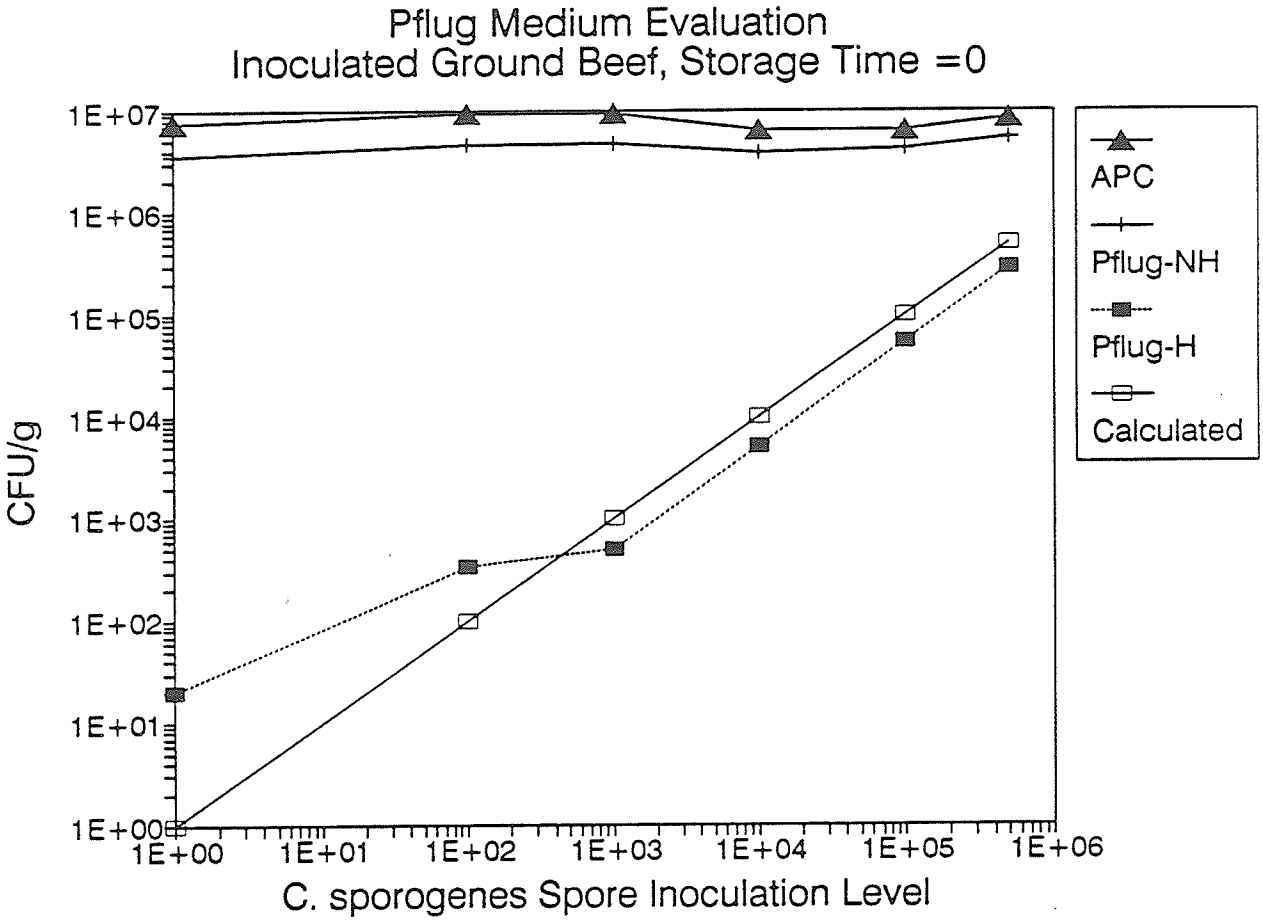
microflora in ground beef monitored with the APC assay are also monitored with the Pflug-NH assay (AnPC).

The clostridial spore assay (Pflug-H) demonstrated good response between the calculated inoculation level and number of spores monitored with the Pflug-H assay. At the 0 and 10^2/g inoculation levels, the spores monitored were greater than the calculated levels (Fig.10). Possible cross contamination between samples may have been the cause of these high values. Between the inoculation levels of 10^3/g and $5x10^5$/g the monitored spores gave a linear response (Fig. 10). The monitored level was approximately 0.25 Log10 lower than the expected (calculated) spore level throughout the linear response (Fig. 10).

This inoculation concentration gradient experiment showed that we can accurately monitor clostridial spores inoculated between the $1x10^3$/g and $5x10^5$/g level using Pflug-H in the presence of normal ground beef microflora. However, total clostridia could not be monitored with Pflug-NH assay because of interference by the high background microflora level in fresh ground beef (Fig. 10). A high percentage of the background microflora appear to be facultative as they grew in both aerobically and anaerobically incubated assays (APC and AnPC).

An experiment was conducted to determine if the Pflug assays could monitor total clostridia and clostridial spores in sterilized ground beef inoculated with C. sporogenes spores. All background microflora in the ground beef would be eliminated in sterilized hamburger prior to inoculation with spores.

Figure 10.


Pflug Medium Evaluation
Inoculated Ground Beef, Storage Time =0
CFU/g
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
1E+00
1E+00
1E+01
1E+02
1E+03
1E+04
1E+05
1E+06
C. sporogenes Spore Inoculation Level
APC
Pflug-NH
Pflug-H
Calculated

5.2.2 Application of Pflug Media for C. Sporogenes Detection in Food

Fresh ground beef was sterilized with a 25 kGy (electron beam) dose. A 550 g portion of the sterilized ground beef was inoculated with 0.400 mL of a $1.4x10^7$/mL C. sporogenes spore suspension. This resulted in a calculated spore concentration of $1.0x10^4$/g in the ground beef. The inoculated ground beef was reground five times at 2° C. Patties were formed and packaged as described in section 4.2. Patties were stored at 20° C in a Atmosbag which was maintained at 10 % oxygen. Initially we had planned that reintroduction of oxygen to a maximum level of only 10 % would be investigated to determine if this would inhibit germination and out growth of inoculated C. sporogenes. Pflug medium was used to evaluate the number of clostridial spores and total clostridia. Non heat-treated stomached samples (Pflug-NH) and Heat-treated stomached samples (Pflug-H) were used to monitor total clostridia and clostridial spores, respectively. The oxygen content inside the package was monitored using a Mocon LF-700D Oxygen Analyzer. Four randomly selected patties were evaluated to determine the homogeneity of the inoculum. The initial number of C. sporogenes spores found in the four random samples were $1.5x10^4$/g ± 0.12.

Inoculated Hamburger Patties

The microbiological and oxygen concentration results for dual and single bag packaged sterilized patties inoculated with C. sporogenes spores, stored at 20° C (10 % oxygen) are shown in Fig. 11 and 12, respectively.

Dual Bag Packaged Patties

In inoculated dual bag packaged patties the APC increased rapidly from no detectable colonies at day 0 of storage to over $3x10^5$/g by day 3 (Fig.11). Between day 3 and day 7 of storage, the APC remained at this level. This result shows that there was some contamination of the hamburger during the inoculation of spores into the sterile ground beef. The contamination level was so low that it was not detected initially, but the contaminating organisms grew rapidly during storage. Despite the contamination the experiment was continued to see if Pflug medium was effective in monitoring C. sporogenes (total and spores) in the presence of this level of contaminating organisms.

The number of white rhizoid colonies typical of C. sporogenes observed on the non heat-treated Pflug medium assay (Pflug-NH) was recorded as total (vegetative and spore) clostridia. Total clostridia numbers were maintained at the $1x10^4$/g inoculation level until the second day. Between day 2 and 3 it increased in parallel with the APC, followed by an increase to over $1x10^7$/g by the seventh day. Many non-rhizoid shaped colonies (Non-typical C. sporogenes), and possible-rhizoid were observed and counted on this non heat-treated assay between day 2 and 7.

The number of white rhizoid shaped colonies typical of C. sporogenes observed on the heat-treated (80° C, 15 min, stomached sample) Pflug medium assay (Pflug-H) was recorded as clostridial spores. Vegetative cells of any kind were destroyed by this heat treatment. Clostridial spores remained at the

$1x10^4$/g inoculation level until day 5 of storage in dual bag packaged patties (Fig. 11). Between day 5 and day 7 the number of clostridial spores increased to approximately $1x10^7$/g.

The oxygen content within the packages was initially 0.20 %. The oxygen content rose to a high of about 0.5 % by day 3 followed by a decrease to undetectable levels by the fifth day. The drop in oxygen concentration within the headspace gas of the nitrogen backflushed patties is probably due to consumption of oxygen by the aerobic organisms present due to contamination.

Single bag Packaged

The results for the inoculated single bag packaged patties are shown in Fig. 12. As with the dual bag packaged samples, the APC of single bag packaged samples increased rapidly from undetectable numbers on day 0 to greater than $1x10^6$/g by day 3 (Fig. 12). Between day 3 and 7 the APC reached a plateau, with a day 7 level of greater than $1x10^7$/g.

The number of typical C. sporogenes colonies observed on the Pflug-NH media assay remained at the $1x10^4$/g calculated inoculation level until day 2 of storage (Fig. 12). Between day 2 and day 7 of storage the number of white colonies on this assay increased and reached a plateau in parallel with the APC.

The results for the total clostridia present in inoculated single bag packaged patties as monitored on the Pflug-H assay were the same as with dual bag packaged patties. The $1x10^4$/g inoculation level was maintained until day 5

of storage, followed by an increase to $1x10^7$/g by day 7 (Fig. 12). This result suggested that the ~ 6.5 % level of O_2 was not able to inhibit spore germination on day 5 of storage at 22° C.

The oxygen content in the package headspace increased rapidly from 0.2% initially to approximately 7% by day 2 and remained near this level until day 7.

Uninoculated Hamburger Patties

The results for the uninoculated dual and single bag packaged patties are shown in Fig. 13 and 14, respectively. No colonies were detected with the Pflug-H (clostridial spore) assay at any time during storage in either packaging condition (results not shown). The APC for the dual bag packaged patties remained at undetectable levels until day 3 when it was over $1x10^4$/g, followed by a modest increase to just over $1x10^5$/g by day 7 (Fig. 13). The results for the Pflug-NH assay (which can be considered AnPC) paralleled the results of the APC. This suggests that the organisms monitored with the APC were in fact facultative. The exception was on day 5 when no colonies were detected. This may have been due to experimental error (miss plating) on this day. The oxygen concentration in the package headspace never exceeded 0.25% during storage.

The results for the uninoculated single bag packaged patties are shown in Fig. 14. The APC increased rapidly from undetectable levels on day 0 to greater

than $1x10^5$/g on day 2. Between day 2 and day 5 the APC decreased to approximately $1x10^2$ /g, followed by an increase to $1x10^6$/g on day 7. The colonies on the Pflug-H assay in general followed the trend set by the APC. The oxygen concentration never reached more than 5% which was somewhat lower than that in the case of the corresponding inoculated samples (Fig. 12).

The oxygen concentration in the headspace gasses in dual bag packaged patties never reached more than 0.1%. Thus the outer barrier bag was very effective. The oxygen concentration in the headspace gases of single bag packaged patties never reached the 10% equilibrium level present surrounding the stored packages, and actually decreased at the later stages of storage. This suggested that the consumption of oxygen by the contaminating organisms present was at a rate greater than the oxygen permeation rate of the inner D-955 film.

The presence of colonies on the non heat-treated Pflug media assay (total clostridia) in uninoculated patties stored in either packaging condition indicated that this assay might not be practical due to interfering colonies which appear similar to the rhizoid shaped C. sporogenes. The similarity between the APC and Pflug-NH assay values over storage in all samples indicated that this assay was not selective enough for use in monitoring inoculated C. sporogenes in radurized ground beef. The minimal contamination (undetectable on day 0) of sterilized ground beef during C. sporogenes inoculation resulted in the growth of background microflora to levels much greater than the $1x10^4$/g C.sporogenes

inoculation level (Fig. 11). The background microflora in radurized ground beef is expected to be even greater due to the much higher initial levels of the microflora present in this experiment (sterilized hamburger which was slightly contaminated during repackaging). Thus there would be even more interfering organisms present in radurized ground beef.

There appeared to be no interference with the clostridial spore Pflug-H assay. In both packaging conditions the calculated inoculation level of $1x10^4$/g was observed initially and upon storage until day 5. Growth in the number of clostridial spores occurred between day 5 and day 7.

The use of Pflug-NH assay to monitor total C. sporogenes in radurized or slightly contaminated "sterilized" ground beef would not be effective due to the interference on the assay of background organisms present. The use of the Pflug-H assay to monitor C. sporogenes spores present in ground beef was demonstrated.

5.2.3 Review of Total C. sporogenes Monitoring Methods

Qualitative or quantitative methods may be used to monitor total C. sporogenes. The qualitative method only determines if C. sporogenes is present or not, and can be used to monitor if there are any surviving C. sporogenes after being subjected to irradiation or thermal treatment. These methods consist of monitoring gas production (bulging or puffing) caused by surviving C. sporogenes in an inoculated food system packaged and incubated.

Aluminum ointment tubes (Robach, 1979), cans (Sofos, 1986b.; Navanugraha, 1973) or vacuum packaged bags (Sofos, 1986b.; Whiting et al., 1985)have been used for packaging in such experiments. Whiting et al. (1985) indicated that monitoring gas production may be a misleading indicator of C. botulinum risk. However, these qualitative methods would not be effective in our inoculation pack situation where C. sporogenes was not expected to be eliminated.

The quantitative methods determine not only if C. sporogenes is present but also at what level. By using heat-treated and non heat-treated assays, spores and total clostridia may be determined. Various media have been used in conjunction with pour plating and anaerobic incubation to monitor C. sporogenes. The media and methods of enumeration used varied between different laboratories. Whiting et al.(1984, 1985, 1986) used Botulinum Assay Media, enumerating number of C.sporogenes by subtracting the CFU/g on uninoculated samples from inoculated samples. Sofos, (1986a) used Peptone-Yeast Extract Glucose, enumerating all CFUs. Molins et al. (1986) used Trypticase Soy Agar, enumerating only rhizoid shaped colonies. DeFreitas et al. (1988a,b.) used Brewers Anaerobic Agar, enumerating the rhizoid shaped colonies. Vareltzis et al. (1984) used Brain-Heart Infusion Agar + 0.05% ferric ammonium citrate + 0.1% sodium sulfite, enumerating the black coloured colonies only.

Figure 11.

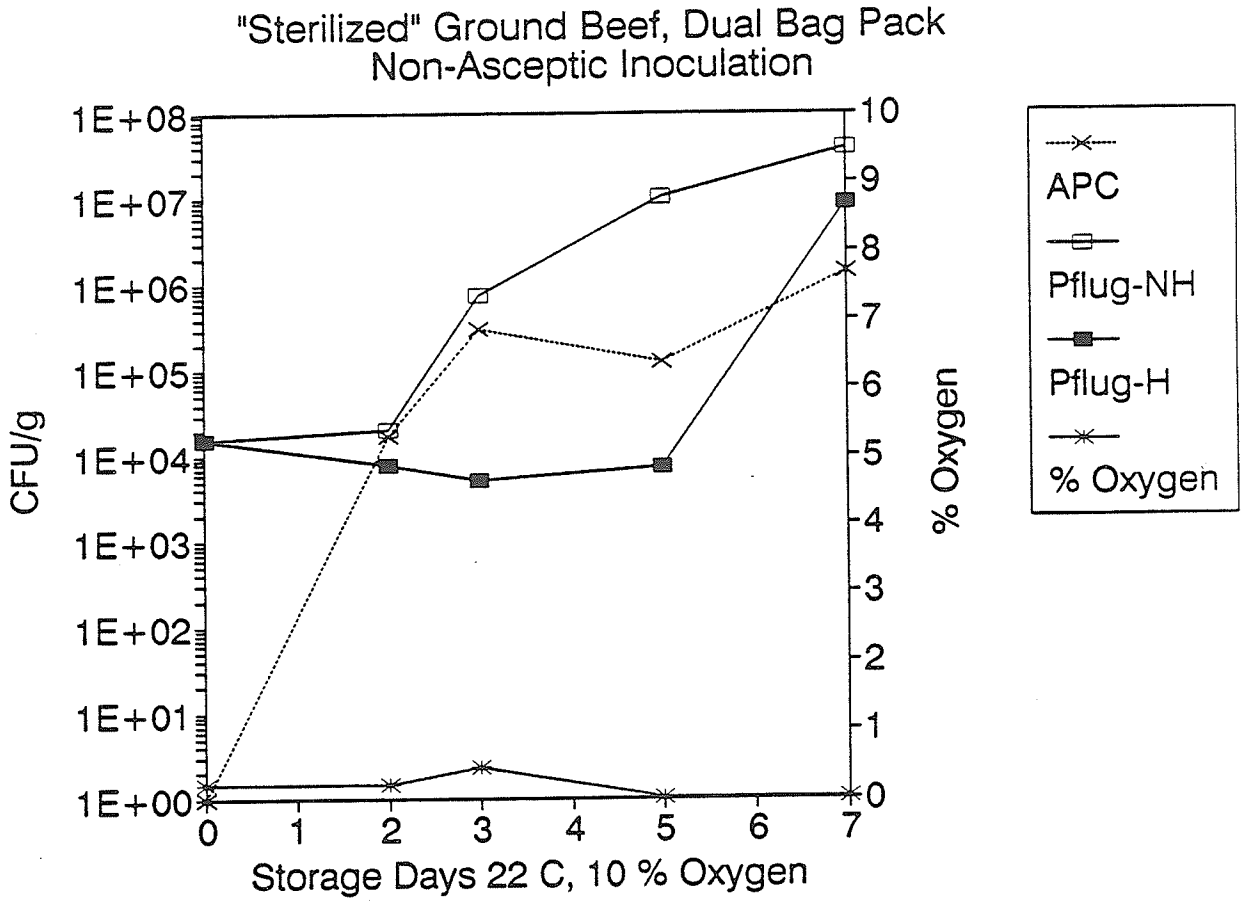

"Sterilized" Ground Beef, Dual Bag Pack
Non-Asceptic Inoculation
CFU/g
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
1E+00
% Oxygen
10
9
8
7
6
5
4
3
2
1
0
0 1 2 3 4 5 6 7
Storage Days 22 C, 10 % Oxygen
APC
Pflug-NH
Pflug-H
% Oxygen

Figure 12.

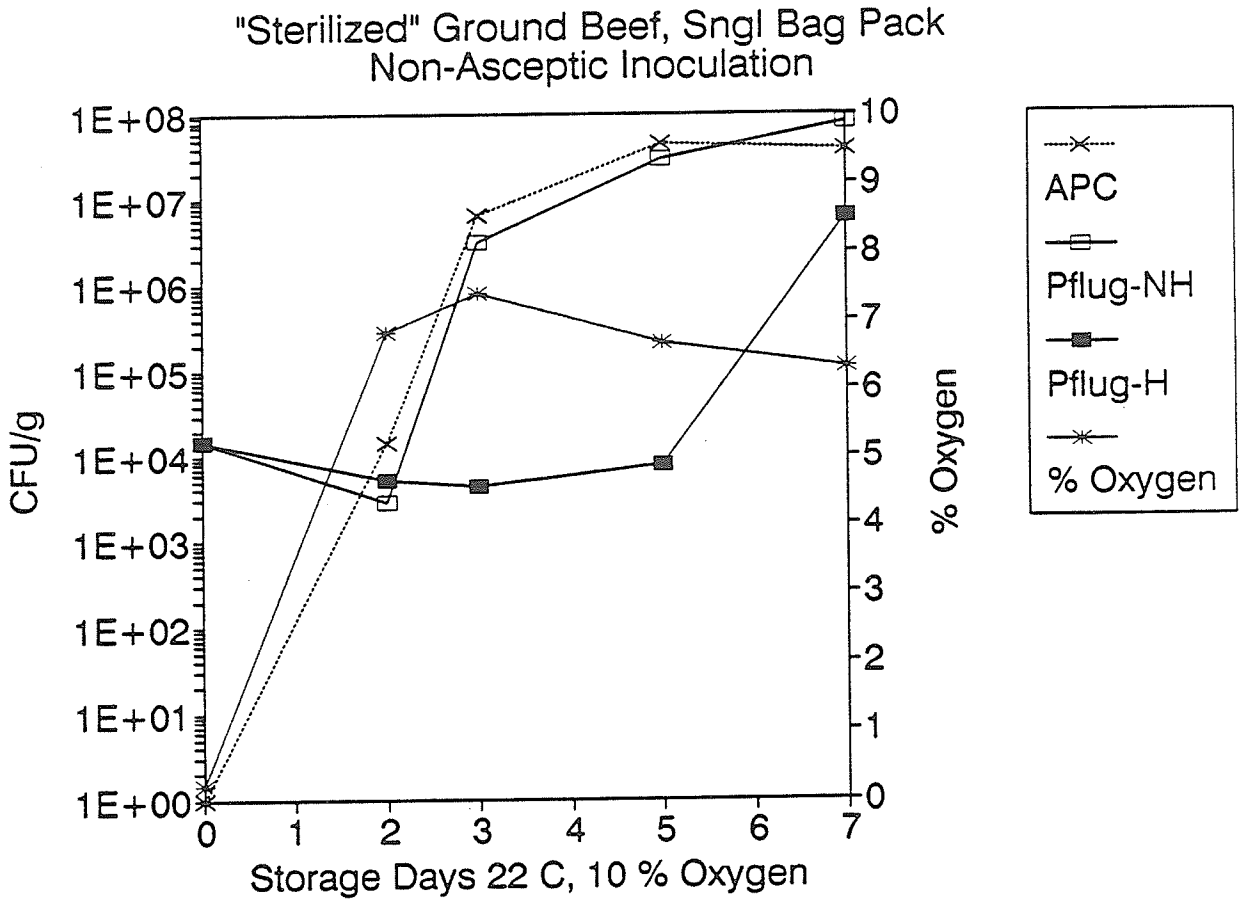

"Sterilized" Ground Beef, Sngl Bag Pack
Non-Asceptic Inoculation
CFU/g
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
1E+00
% Oxygen
10
9
8
7
6
5
4
3
2
1
0
0 1 2 3 4 5 6 7
Storage Days 22 C, 10 % Oxygen
APC
Pflug-NH
Pflug-H
% Oxygen

Figure 13.

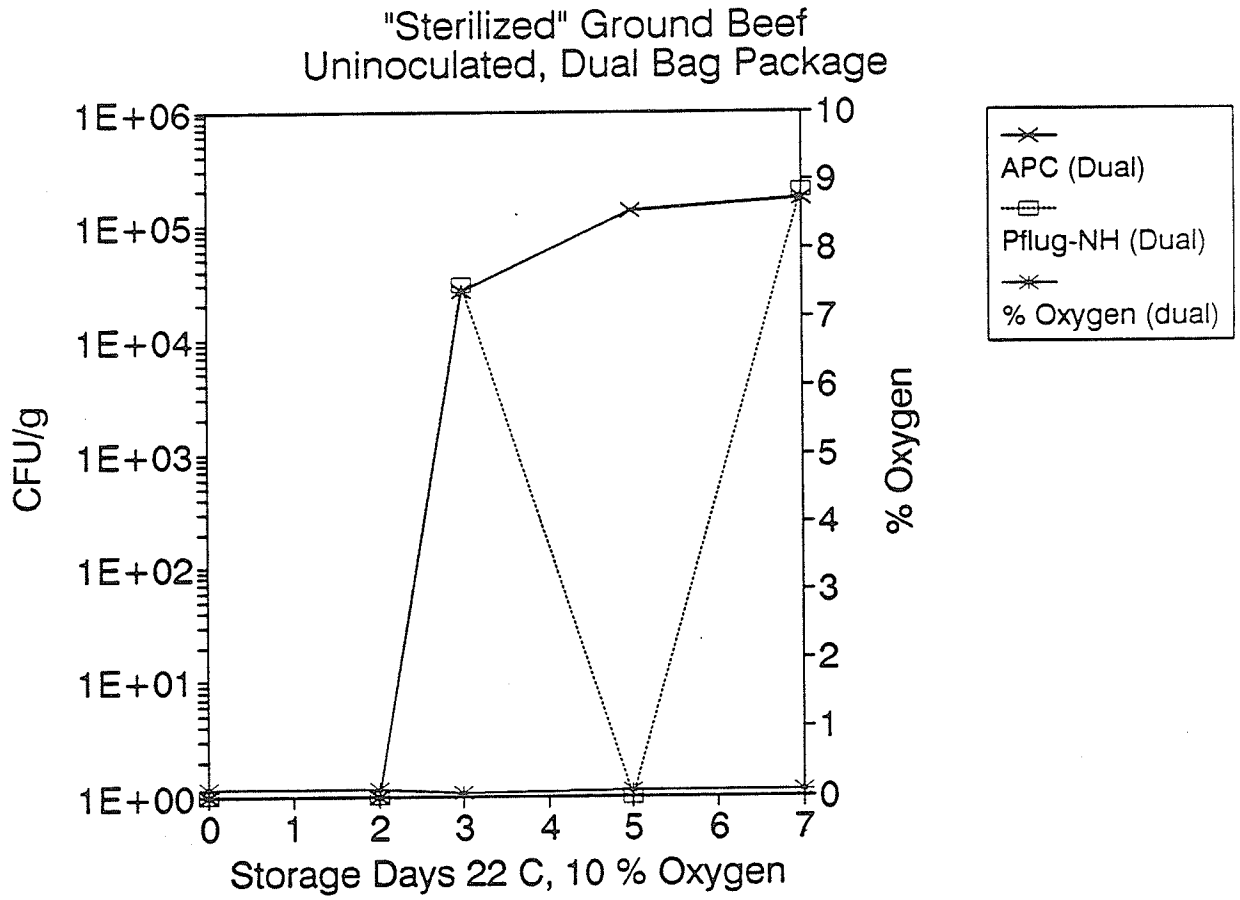
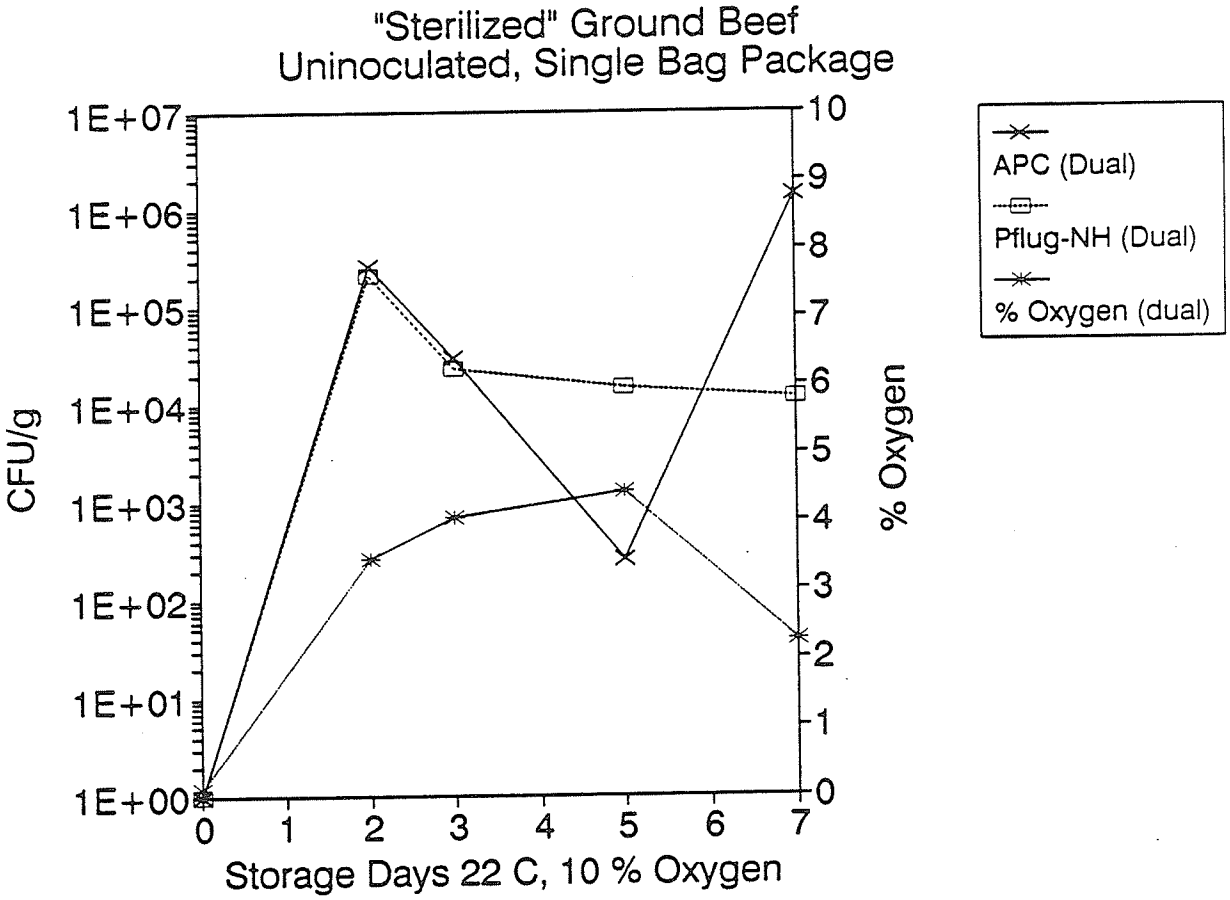

"Sterilized" Ground Beef
Uninoculated, Dual Bag Package
CFU/g
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
1E+00
% Oxygen
10
9
8
7
6
5
4
3
2
1
0
0 1 2 3 4 5 6 7
Storage Days 22 C, 10 % Oxygen
APC (Dual)
Pflug-NH (Dual)
% Oxygen (dual)

Figure 14.


"Sterilized" Ground Beef
Uninoculated, Single Bag Package
CFU/g
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
1E+00
% Oxygen
10
9
8
7
6
5
4
3
2
1
0
0 1 2 3 4 5 6 7
Storage Days 22 C, 10 % Oxygen
APC (Dual)
Pflug-NH (Dual)
% Oxygen (dual)

Pivinick et al. (1970) used Wynne broth and three tube most probable number (MPN) tables to enumerate number of C. sporogenes. All of the food systems studied by the above listed researchers were either, thermally processed (DeFreitas et al., 1988a,b.; Molins et al., 1986; Sofos, 1986a.; Vareltzis et al. 1984; Whiting et al., 1985, 1986) or previously treated by radiation (Pivnick et al., 1970; Whiting et al., 1984). These treatments, reduced or eliminated interfering background organisms. Even with these treatments some researchers had to abort experiments when background microflora levels increased to greater than the inoculated C. sporogenes levels.

After reviewing the above methods utilized to monitor C. sporogenes it was decided the two best possible solutions to the problem of monitoring the inoculated total clostridia were:

1) Increase inoculation level to greater than the background level on Pflug-NH assay.

2) Develop a selective and/or differential assay, so that total clostridia may be monitored at the present inoculation level.

Since the former would require an unrealistically high level of spores for inoculation and it would require an unmanageable number of spores it was decided to pursue the option 2) of a selective and/or differential assay to monitor total (vegetative and spores) Clostridium sporogenes at about $1x10^4$/g level in fresh ground beef with an APC of greater than $1x10^6$/g.

5.2.4 Development of a Selective and/or Differential Total C. sporogenes Assay

An assay which is selective and/or differential for total C. sporogenes must allow the enumeration of vegetative and spore forms in a sample with high background microbial levels. As indicated in Section 3, the background predominant microflora of stored fresh ground beef includes members of: pseudomonas, micrococcus, lactobacillus, genera, enterobacteriaceae, and yeast. The predominant microflora of radurized stored ground beef includes members of: Micrococci such as Lactobacilli, Leuconostoc, and pediococci (LLP Group),and streptococcus, yeast, and B. thermosphacta.

In the method utilized by Vareltzis et al. (1984) which includes ferric ammonium citrate and sodium metabisulfite, C.sporogenes reduces the sulfite to sulfate allowing the ferric ions to react with it, resulting in a black colored precipitate (black coloured colonies). The reaction producing black C. sporogenes colonies make the media discriminative, and the sodium metabisulfite in the media also makes it some what selective to some groups of organisms.

An experiment was undertaken to determine if these two chemicals could be added to Pflug media to allow for the differential enumeration of C. sporogenes. It was decided to use Pflug media as the base as it proved to be most effective in recovering radiation stressed C. sporogenes (Shamsuzzaman, 1989). Total C. sporogenes (non heat-treated), and C. sporogenes spores (heat-treated) inoculated into ground beef were subjected to a radurization dose of 2.5 kGy

(gamma). There were three samples (20 ± 0.5 g) of ground beef for each of the three sample treatments. The sample treatments were:

1) No inoculation, no irradiation (Control)

2) No inoculation, 2.5 kGy

3) $5x10^5$/g inoculation, 2.5 kGy

Each 20 g portion of inoculated ground beef was inoculated with 0.741 mL of a $1.4x10^7$/mL C. sporogenes spore suspension and mixed with a sterile fork. This resulted in the calculated pre-radurization C. sporogenes inoculation level of $5x10^4$/g. The modified Pflug medium prepared as in Section 4.2 was modified by adding 0.1 % ferric ammonium citrate and 0.1 % sodium metabisulfite to Pflug's media prior to autoclaving.

The results for the comparison of the Pflug and Modified Pflug medium for the enumeration of inoculated C. sporogenes (total and spores only) and interfering natural microflora in radurized and control ground beef are shown in Table 5.

Assay for Non-heat-treated Total Count

The results in Table 5 show that there was greater than a 2 Log10 reduction in APC when samples were irradiated to 2.5 kGy (gamma). The results for the total clostridia (Non heat-treated) Pflug and Modified-Pflug assay both showed 2.5 Log10 reductions in "All" colonies observed from 8.8 and $6.2x10^6$/g to 3.1 and $2.1x10^4$/g respectively. These are in fact monitoring the AnPC group of

organisms and show the level expected in radurized and control samples of ground beef. The addition of the modifying agents (Modified-Pflug media) appear to result in only minor reductions in the organisms observed. There were no typical C. sporogenes (white rhizoid shaped) colonies observed in any of the uninoculated samples with either media. There were $2.6x10^4$/g and $1.9x10^4$/g typical C. sporogenes (white rhizoid shaped) colonies observed with Pflug and Modified-Pflug media, respectively in the inoculated, irradiated hamburger. This was the expected level of C. sporogenes after a 2.5 kGy dose was applied to the $5x10^4$/g initial calculated inoculation level. It was noted that the 5:1 ratio of "All" colonies/typical C. sporogenes white colonies as observed in this experiment was the maximum which could be visually discriminated. This ratio relates to visually discerning 50 rhizoid colonies on a plate with 250 total colonies. Since this maximum ratio was already reached at this time (0 storage time) and large increases in background microflora would be expected over storageof ground patties (section 5.2.1 and Niemand, 1983) these results confirm that Pflug medium would not be effective in monitoring total C. sporogenes at this inoculation level. Increasing the inoculation level was again ruled out as it would create an unrealistic contamination situation and required the propagation of large numbers of these spores.

The Modified-Pflug medium showed an estimated $2x10^3$ black colonies/g in the inoculated samples, this was based on the average of three samples (0, 0, and $1.4x10^4$/g). There were no indigenous black colonies present in the

uninoculated samples. The recovery of these black colonies is a 1 Log10 level lower than recovery of C. sporogenes by visually discerning colony shape ($1.9x10^4$/g). Most of the inoculated C. sporogenes appeared as white rhizoid colonies. It would require a majority of the $1.9x10^4$/g white rhizoid colonies to appear black for the medium to be considered effective as a differential media for C. sporogenes. Even with the reduced and variable recovery rate this non heat-treated assay showed the potential of using the number of black coloured colonies present as a method of monitoring total C. sporogenes with high levels of background organisms as found in radurized ground beef. Therefore, further investigation of this method using modified Pflug's media was warranted.

Assay for Heat-Treated Spore Count

The results for the heat treated assays, which monitor spores only are also shown in Table 5. No colonies were observed with either the Pflug or Modified-Pflug assays for uninoculated samples. This shows that there are no interfering spores present in fresh or radurized ground beef. All the colonies observed on Pflug medium plates were typical rhizoid shaped colonies, yielding an average value of $1.8x10^4$/g. The Modified-Pflug medium monitored $1.4x10^4$ black colonies /g. This is very close to the $1.8x10^4$/g typical C. sporogenes recorded with the Pflug Medium. There were $0.4x10^4$/g white rhizoid typical C. sporogenes colonies on Modified-Pflug in addition to the black colonies observed. Thus, a vast

majority of C. sporogenes produced black colonies on the Modified-Pflug medium. Thus, C. sporogenes spores can be more effectively monitored with a heat-treated sample compared to a non heat-treated sample using Modified-Pflug medium.

Table 5. Inoculation Pack Study C. sporogenes Recovery on Various Media Fresh Ground Beef Day 0 of Storage

Inoculated			No	No	Yes
Dose (kGy)			0	2.5	2.5
Media	Heat Treat	Colony Type	CFU/g		
APC	No	All	$1.5x10^7$	$3.8x10^4$	$9.5x10^4$
Pflug	No	All*	$8.8x10^6$	$3.1x10^4$	$1.5x10^5$
Pflug	No	White Rhizoid	0	0	$2.6x10^4$
Mod.-Pflug	No	All*	$6.2x10^6$	$2.1x10^4$	$9.1x10^4$
Mod.-Pflug	No	White Rhizoid	0	0	$1.9x10^4$
Mod.-Pflug	No	Black	0	0	$2x10^3$ †
Pflug	Yes	All	0	0	$1.8x10^4$
Pflug	Yes	White Rhizoid	0	0	$1.8x10^4$
Mod.-Pflug	Yes	All	0	0	$1.9x10^4$
Mod.-Pflug	Yes	White Rhizoid	0	0	$0.5x10^4$
Mod.-Pflug	Yes	Black	0	0	$1.4x10^4$

* A type of AnPC

† Average of 3 samples (0, 0, 1.4x10 4/g)

5.2.5 Evaluation of Selective and/or Differential Assays for Total C. sporogenes (Non heat-Treated)

Further work was done to evaluate other potential selective / differential media for monitoring C. sporogenes. A total C. sporogenes assay (non heat-treated) was especially required as the Pflug and Modified-Pflug both proved effective for monitoring spores (heat-treated) in ground beef samples when there were no indigenous spores present to interfere with these assays.

The use of Sulfite Cycloserine (SC) Agar for the enumeration of C. perfringens in food is part of the recommended MFA-23 method according to HPB (1982). The SC agar was made by preparing SFP agar and adding 1mL/100mL of agar to a 4% filter sterilized solution of d-Cycloserine. The SFP agar prepared according to the manufacturers instructions contains 0.1% ferric ammonium citrate and 0.1% sodium metabisulfite, so C. perfringenes will produce black colonies. Harmon and Duncan (1984) state that C. sporogenes will interfere with this assay and produce similar black colonies, thus suggesting that this medium could be used to monitor C. sporogenes and may be more effective than the Modified-Pflug medium as it is not only discriminative (produces black colonies) but also contains the antibiotic d-Cycloserine so it may also be more selective. This along with the fact that it is a more enriched medium than Modified-Pflug suggests that it may improve the recovery of C. sporogenes.

Table 6. Enumeration of C. sporogenes Spore Suspension on Various Media

Media	Heat Treated	Not Heat Treated
	CFU/mL	
Pflug	$1.3x10^7$a	$1.1x10^7$a
Modified-Pflug	$2x10^5$Eb	$<5x10^2$b
SFP	$<5x10^2$b	$<5x10^2$b

a White Rhizoid Colonies
b Black Colonies

The results from an experiment comparing the recovery of C. sporogenes spores from the combined batch #1-4 stock suspension are shown in Table 6. The concentrations of C. sporogenes in the combined batch when monitored with Pflug medium were $1.3x10^7$/mL and $1.1x10^7$/mL for the heat-treated and non heat-treated assays, respectively. The Modified-Pflug medium and SFP medium were ineffective in enumerating C. sporogenes via black coloured colonies (Table 6). The poor performance of Modified-Pflug medium in this experiment (even though preliminary results from Table 5 indicated some potential for the use of this medium) showed that further modifications to this medium or another type of medium must be investigated due to lack of consistent recovery. The poor performance of the SFP to monitor C. sporogenes was a surprise since Harmon and Duncan (1984) stated it would produce a black colony on SC media. The absence of Cycloserine (due to unavailability at time of the experiment) from the SC medium as directed by MFA-23 (HPB, 1982) meant that the media was only composed of the SFP medium. This lack of Cycloserine may be the reason for the poor recovery, although this was not believed to be so initially. This was demonstrated in a later experiment (Table 9) where addition of Cycloserine to SFP medium still resulted in poor recovery of C. sporogenes as black colonies. Next we considered the addition of the three supplements to the SFP medium

that Polvino and Bernard (1982) found to be effective in increasing the recovery of C. sporogenes in Pflug medium may be effective in increasing the recovery on SFP medium and were investigated.

The results described in Table 7 compare the effectiveness of various media on the recovery of C. sporogenes spores using the heat-treated and non heat-treated assay methods was done to monitor the clostridial spores and total clostridia, respectively. The Pflug + Cycloserine and Modified-Pflug + Cycloserine media were evaluated to determine what the effect of adding Cycloserine to the media would be on the recovery of C. sporogenes. A 4 % filter sterilized solution of Cycloserine was added at a rate of 1 mL/100mL of tempered autoclaved agar as indicated. The Modified-Pflug#2 medium was prepared as Modified-Pflug medium with the exception that the ferric ammonium citrate and sodium metabisulfite were added after autoclaving as filter sterilized solutions. This medium was evaluated as there was concern that the effectiveness of the two added chemicals to permit the formation of black colonies by C. sporogenes may be hindered if exposed to the heat of autoclaving. The SFP + three supplements were added as follows: SFP medium was prepared according to the manufacturers instructions, the three filter sterile supplements used with the Pflug medium were added to the tempered autoclaved SFP medium. The supplements were: 12.5% Sodium Thioglycolate, 50% Dextrose and 10% Sodium Bicarbonate. The supplements were added at a concentration rate of 1 mL/100 mL agar.

The results in Table 7 show that the number of C. sporogenes monitored with the Pflug-H and Pflug-NH assays were 1.0×10^7/mL and 6.9×10^6/mL, respectively, for the C.sporogenes spore suspension. The greater recovery using the heat-treated assay as opposed to the non heat-treated assay was expected as Shamsuzzaman (1989) found that heat activation of spores results in improved germination of spores. The Pflug + Cycloserine medium demonstrated slightly reduced recovery of C. sporogenes compared to Pflug medium alone. The heat-

treated and non heat-treated Pflug + Cycloserine assays monitored $8.6x10^6$/mL and $6.4x10^6$/mL, respectively, compared to $1.0x10^7$/mL and $6.9x10^6$/mL for the Pflug medium alone. The SFP + three supplements medium demonstrated slightly increased recovery of C. sporogenes compared to Pflug medium. The heat-treated and non heat-treated SFP + three supplements assays monitored $1.2x10^7$/mL and $1.0x10^7$/mL black coloured colonies, respectively. The increased recovery of spores when an assay uses a heating process in which heat activates spores leading to improved germination is also evident with this medium. The Modified-Pflug, Modified-Pflug #2, and Modified-Pflug + Cycloserine media all recorded $<5x10^2$ black colonies/mL, thus showing that delaying the addition of the black colony producing agent until after autoclaving did not improve recovery of spores, and that addition of Cycloserine did not improve the recovery.

Table 7. C. sporogenes Spore Suspension Enumeration on Various Media

Media	Heat Treated	Not Heat Treated
	CFU/mL	
Pflug	$1.0x10^7$†	$6.9x10^6$†
Pflug + Cycloserine	$8.6x10^6$†	$6.4x10^6$†
SFP + 3 Supplements	$1.2x10^7$‡	$1.0x10^7$‡
Modified-Pflug	$< 5x10^2$‡	$< 5x10^2$‡
Modified-Pflug #2	$< 5x10^2$‡	$< 5x10^2$‡
Modified-Pflug + Cycloserine	$< 5x10^2$‡	$< 5x10^2$‡

† Rhizoid Shaped Colonies
‡ Black Colonies

The effectiveness of SFP + three supplements medium was demonstrated by the results in Table 7. The use of this medium (and possibly SFP + Cycloserine + three supplements) appeared to be a potential solution to monitoring total clostridia in ground beef. The effect of the natural microflora in ground beef on these assays remained to be determined to see if there were

any interfering background organisms and producing black colonies. The results are described below.

5.2.6 Background Microflora in Ground Beef

An experiment was undertaken to determine the type and extent of interference from the background microflora in ground beef using SFP + three supplements. Ground beef was dual bag packaged and stored for 5 days at 2° C to allow the natural microflora to increase, as a product with a high background microflora was desired. The media tested were prepared as stated earlier.

Table 8. Evaluation of Background Microflora of: Ground Beef, 5 Day Vacuum Packed at 2° C, Enumeration on Various Media

Media	Heat Treated	Not Heat Treated
	CFU/g	
	(WHITE COLONIES)	
SFP + Cyclo + 3 Supp. (SC+3)	< 10	1.2 X 10^7E
SFP + 3 Supplements	< 10	1.2 X 10^7E
SFP + Cycloserine (SC)	< 10	1.1 X 10^7E
Pflug	< 10	2.8 X 10^7
	(BLACK COLONIES)	
SFP + Cyclo + 3 Supp. (SC+3)	< 10	< 10
SFP + 3 Supplements	< 10	< 10
SFP + Cycloserine (SC)	< 10	3.3 X 10^2E
Pflug	NA	NA

NA = Not Applicable
E = Estimate

The results of the enumeration of white and black colonies produced by the background microflora in ground beef on various media are shown in Table 8. There were no recorded colonies, (white or black) with any of the media tested when the heat treated assay was used. With the dilution levels used this insures

that there were <10 colonies/g of ground beef. Thus there are no interfering spores present in this ground beef sample.

The number of white colonies observed on all the media tested with a non heat-treated assay were similar. The three media with SFP all recorded an estimated $1x10^7$/g, while the Pflug medium had $2.8x10^7$/g of white colonies. This means that the number of white colonies enumerated was not affected by the variations in the media tested.

There were no interfering black colonies present when the non-heat-treated assays were used with SFP + Cycloserine + three supplements (SC+3), or SFP + three supplements media (Table 8). There were an estimated $3.3x10^2$/g of black colonies as monitored by the SFP + Cycloserine (SC) medium. The lack of black colonies in the first two media and minimal presence of black colonies monitored with the last media indicated that the interference by the natural microflora in the ground beef may not cause a problem in discerning the inoculated spores in ground beef. No black colonies were observed with the Pflug medium, as expected, since it does not contain the agents which allow for the production of black colonies.

5.2.7 Enumeration of C.sporogenes Spores on Various Media

The recovery of C. sporogenes spores using the heat-treated assay with four different media described above and Table 8 was further investigated. The number of black and white rhizoid colonies was recorded. The results are shown in Table 9.

Table 9. Enumeration of Heat Treated C. sporogenes Spore Suspension on Various Media

Media	Colony Colour (Shape) CFU/mL Black	White Rhizoid	(W/B) %
SFP + Cycloserine + 3 Supp.(SC+3)	$1.6x10^7$	$1.2x10^6$	7.5
SFP + 3 Supplements	$1.6x10^7$	$1.3x10^6$	8.1
SFP + Cycloserine (SC)	$6.5x10^2$E	<10	NA
Pflug	NA	$1.2x10^7$	NA

NA Not Applicable
E Estimate

The SFP + Cycloserine + 3 Supplements (SC+3) and SFP + 3 Supplements media both had similar recovery of C. sporogenes spores (in suspension) using heat-treated spores. They both showed $1.6x10^7$ black colonies/mL and $1.2x10^6$ white rhizoid colonies/mL. This resulted in a white rhizoid/black colony ratio of 8% for both. The SFP + Cycloserine (SC) medium recorded only an estimated $6.5x10^2$ black colonies/mL, and no white rhizoid colonies were observed. The Pflug medium monitored $1.2x10^7$ white rhizoid colonies/mL. These results show that SFP + 3 Supplements (with or without Cycloserine) recover C. sporogenes spores more effectively. The addition of the three supplements to the SFP media was essential to good recovery. Further addition of Cycloserine did not affect the recovery. So the SC media (of HPB,

1982) had to be modified to SC+3 to be effective in our application of monitoring C. sporogenes spores.

The presence of the ~8% white rhizoid colonies in the SFP + 3 Supplement media (with and without Cycloserine) raised concerns that perhaps there was contamination in the C. sporogenes inoculum. Representative black and white rhizoid colonies were picked and serially diluted. The various dilutions were plated on the SFP + Cycloserine + 3 Supplements (SC+3) medium. The results from all the colonies selected were approximately 8% white rhizoid, with the remainder being black colonies. This indicates that with the assay as stated, 92% of the C. sporogenes colonies will be black. Further work to increase the percentage of black colonies was ruled out as the assay was already more effective than the Pflug medium assay.

5.2.8 Enumeration of Radiation Stressed Spores

Shamsuzzaman (1989) had found that the Pflug medium was more effective in recovering radiation stressed C. sporogenes spores. An experiment was conducted to determine if the SFP-based medium would lose its effectiveness compared to the Pflug medium in recovering radiation stressed C. sporogenes spores. Two samples from the C. sporogenes spore suspension were subjected to a gamma dose of 2.5 kGy.

The results for the non heat-treated assay using SFP + Cycloserine + 3 Supplements (SC+3) and Pflug media are shown in Table 10. The average

recovery of the two samples with the SFP + Cycloserine + 3 Supplements (SC+3) and the Pflug media were $4.6x10^6$/mL and $3.2x10^6$/mL, respectively. These results show that the effectiveness of this SFP-based medium compared to the Pflug medium was also applicable to the radiation stressed spores (as well as with no stressed spores as seen before).

Table 10. Enumeration of Non Heat-Treated, 2.5 kGy Gamma Stressed C. sporogenes Spore Suspension on Various Media

Media	Sample "A"	Sample "B"	Average
	CFU/mL		
SFP + Cyclo + 3 Supp.(SC+3)*	$4.4x10^6$	$4.8x10^6$	$4.6x10^6$
Pflug **	$3.1x10^6$	$3.2x10^6$	$3.2x10^6$

* Black Colonies
** White (Typical Rhizoid Shape)

5.2.9 Assessment of SC+3 Media for Inoculation Pack Study

An inoculation pack study was conducted to determine if fresh ground beef inoculated with varying amounts of C. sporogenes spores could be monitored accurately with the SC+3 medium. Non heat-treated stomached samples (SC+3-NH) were used to monitor total clostridia. C. sporogenes spore inoculation levels were: 0, 10^1, 10^3, 10^4, 10^5, and $5x10^5$/g. White non-rhizoid shaped colonies on the SC+3-NH assay are given as AnPC in Fig. 15. The average AnPC for the ground beef samples was $6.3x10^6$/g (Fig. 15) using the SC+3 medium which was

close to the AnPC of $4.4x10^6$/g using Pflug medium (Fig. 10). Thus the background microflora used in this experiment at different C. sporogenes inoculation levels are similar to that used in section 5.2.2.

The total clostridia assay (SC+3-NH) demonstrated good response between the calculated inoculation level and the number of clostridia monitored with the SC+3 assay (Fig. 15). Only at the 0/g inoculation levels were the clostridia monitored greater than the calculated levels. Possible contamination through experimental error or the presence of natural microflora in the ground beef, which could produce black colonies with the SC+3 assay, was believed to be responsible for the higher than expected value. At the $1x10^1$/g inoculation level no black colonies were observed. This was a 1 Log_{10} lower response than expected. However, between the $1x10^3$/g and $5x10^5$/g inoculation levels the monitored total clostridia gave a linear response at a level approximately 0.25 Log10 lower than the expected (calculated) spores (Fig. 15). This was the same reduction in the observed clostridia as shown in Figure 10 with the Pflug-H medium monitoring spores only. The SC+3 medium has been shown to be more effective than the Pflug medium in monitoring spores (section 5.2.7) and radiation stressed total clostridia (section 5.2.8), and can accurately monitor total clostridia inoculated between the $1x10^3$/g and $5x10^5$/g inoculation levels. The total clostridia were selectively monitored (black colonies) in ground beef with high background microflora levels (AnPC $6.3x10^6$/g). The Pflug media could only monitor clostridial spores between these levels of inoculation, as the total

clostridia (Pflug-NH) assay was interfered with by the high AnPC present in ground beef.

5.2.10 Inoculation Pack Study, Final Medium Modification

An inoculation pack study with dual bag packaged irradiated (2.3 kGy electron beam) and unirradiated control patties of fresh ground beef was conducted. Microbiological evaluations included: APC, total clostridia (SC+3-NH), and clostridial spores (SC+3-H) assays. Storage temperatures included: 2°-4°, 10°, 15°, and 20°C.

The results for the dual bag packaged irradiated (2.3 kGy) and unirradiated control patties are shown in the figures indicated: 2°-4°C (Fig. 16), 10°C (Fig. 17), 15°C (Fig. 18), and 20°C (Fig. 19). Concern for the effectiveness of the total clostridial (SC+3-NH) assay was raised due to the results in figures 16 to 20. In an inoculated patty, the total clostridia (SC+3-NH) monitored should be equal to or greater than the clostridial spores alone (SC+3-H). It was observed that at 10°, 15°, and 20°C storage temperatures (Figs. 17, 18, 19), on the first day of storage evaluated after day 0 (day 5, 5, and 3 respectively on the figures), no total clostridia were monitored in irradiated patties while the clostridial spores monitored remained at the initial inoculation level ($1x10^4$/g). At these same evaluation days the total clostridia and clostridial spores in unirradiated control patties were similar at the initial inoculation level of approximately $1x10^4$/g. The total clostridia and clostridial spores for the 2°-4°C

stored irradiated and unirradiated control patties were similar at approximately $5x10^3$/g, a slight drop from the initial inoculation level of approximately $1x10^4$/g (Fig. 16).

These results indicate that at the 10°, 15°, and 20°C storage temperatures, irradiated patties are showing inappropriate or poor recovery on the total clostridia (SC+3-NH) assay compared to the clostridial spore (SC+3-H) assay. The problem does not occur with the unirradiated control patties or any patties stored at 2°-4°C. Evaluations conducted at day 10 of storage for these samples stored at 10°, 15°, 20° C did not show this poor recovery with the total clostridia assay, but there was still a concern that the assay required modifications. In radurized and unirradiated vacuum packaged meat, lactic acid bacteria (LAB) will become the predominant microflora upon storage. The percentage of LAB contributing to the APC or AnPC as monitored could not be determined as no selective medium was used. From literature it was found that LAB are facultative organisms and thus would be able to grow on the anaerobically incubated SC+3-NH assay and probably are the major component of the AnPC or white colonies on this assay. They would not be a factor on the SC+3-H assay as they would be destroyed by the heat treatment. The production of lactic acid by these organisms, while they grow in the SC+3 medium with the non heat-treated assay, may cause the pH of the medium to be lowered. The pH of the media was monitored after incubation in the subsequent experiments.

Figure 15.

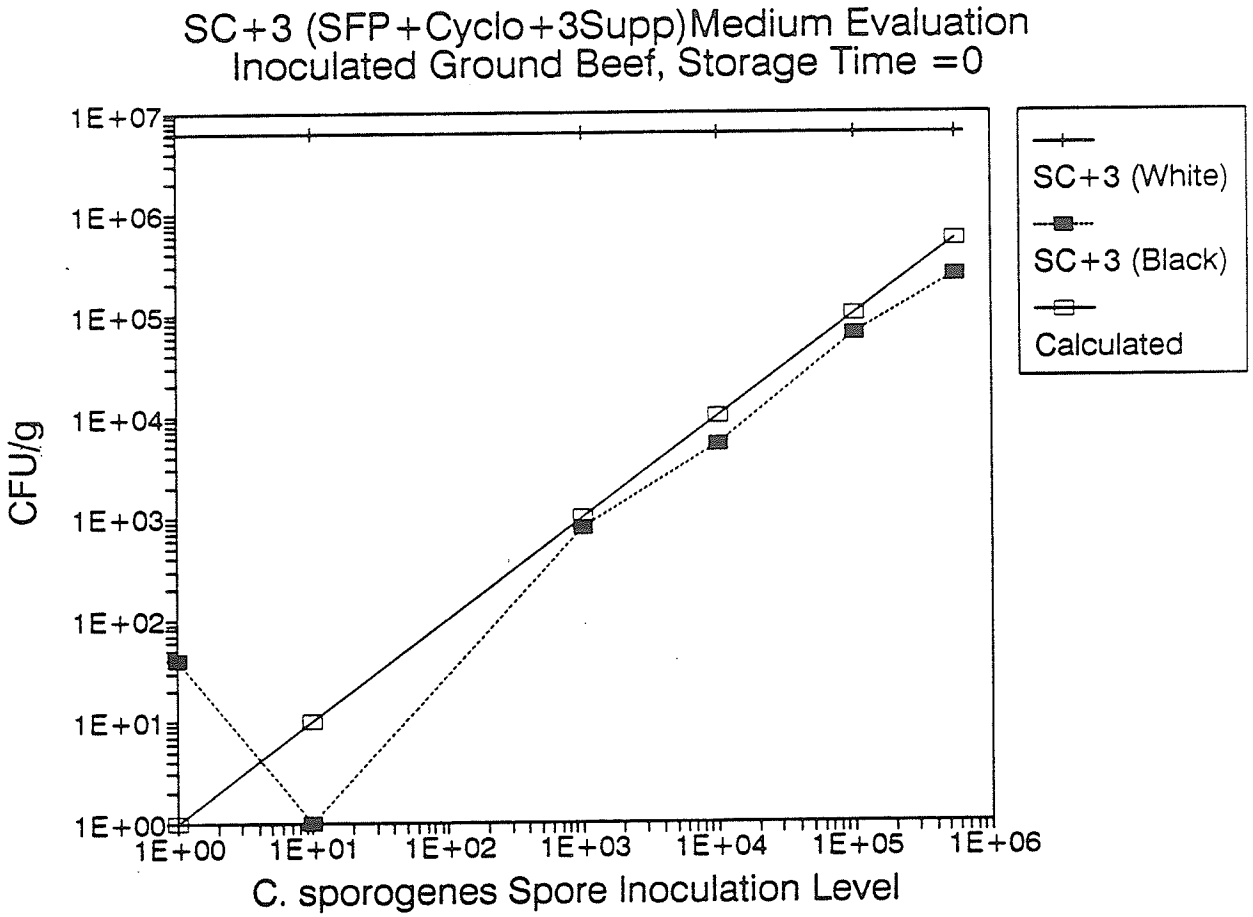

SC+3 (SFP+Cyclo+3Supp)Medium Evaluation
Inoculated Ground Beef, Storage Time =0
CFU/g
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
1E+00
1E+00
1E+01
1E+02
1E+03
1E+04
1E+05
1E+06
C. sporogenes Spore Inoculation Level
SC+3 (White)
SC+3 (Black)
Calculated

Figure 16.

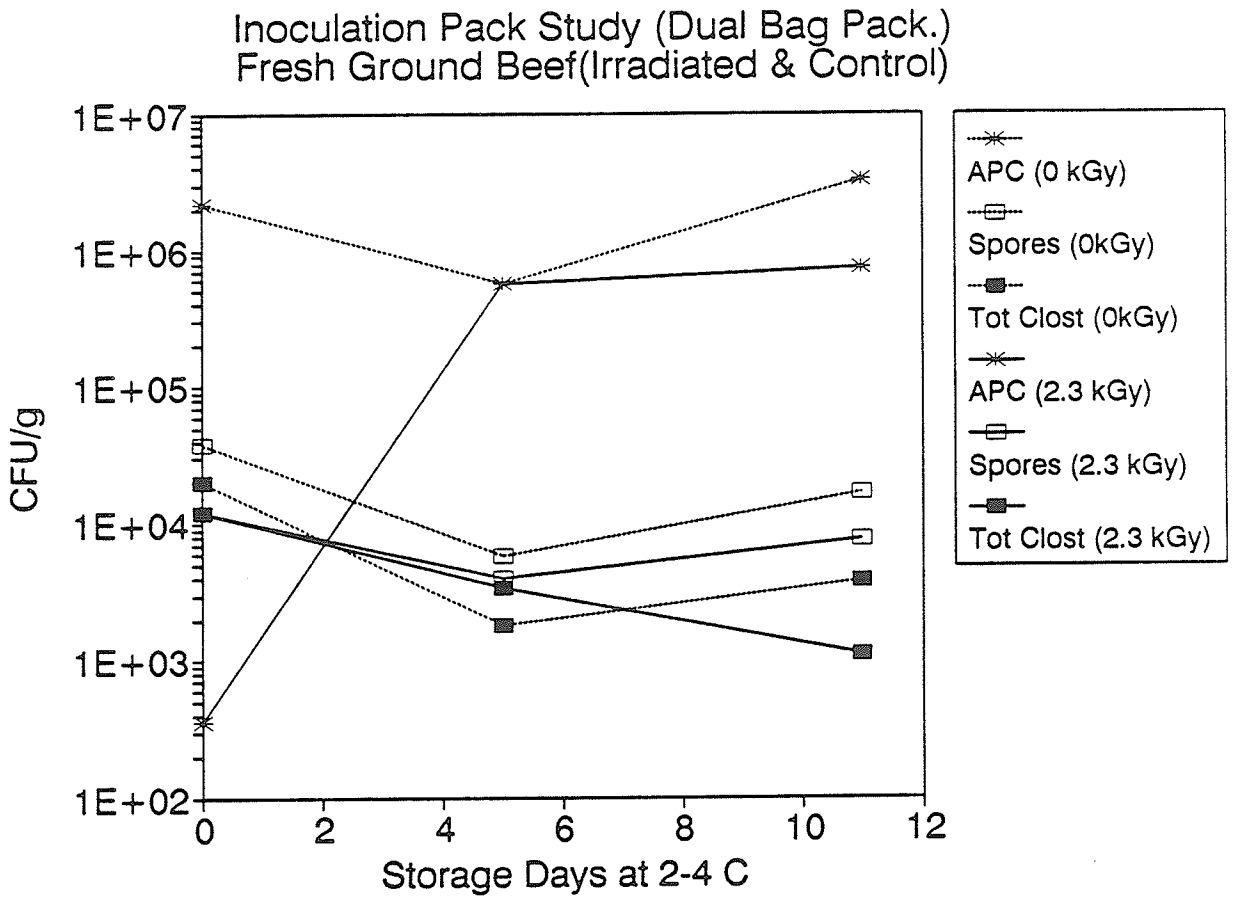

Inoculation Pack Study (Dual Bag Pack.)
Fresh Ground Beef(Irradiated & Control)
CFU/g
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
0
2
4
6
8
10
12
Storage Days at 2-4 C
APC (0 kGy)
Spores (0kGy)
Tot Clost (0kGy)
APC (2.3 kGy)
Spores (2.3 kGy)
Tot Clost (2.3 kGy)

Figure 17.

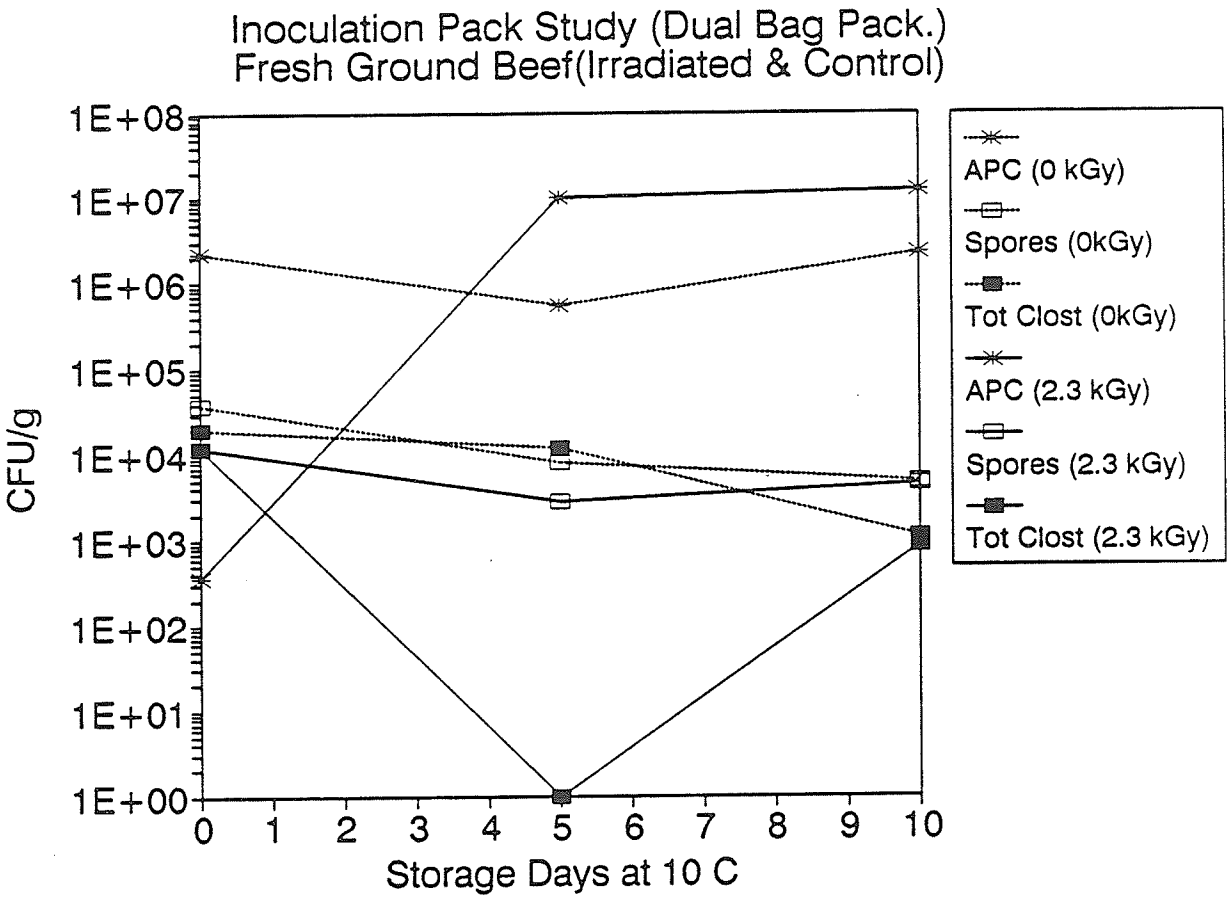

Inoculation Pack Study (Dual Bag Pack.)
Fresh Ground Beef(Irradiated & Control)
CFU/g
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
1E+00
0 1 2 3 4 5 6 7 8 9 10
Storage Days at 10 C
APC (0 kGy)
Spores (0kGy)
Tot Clost (0kGy)
APC (2.3 kGy)
Spores (2.3 kGy)
Tot Clost (2.3 kGy)

Figure 18.

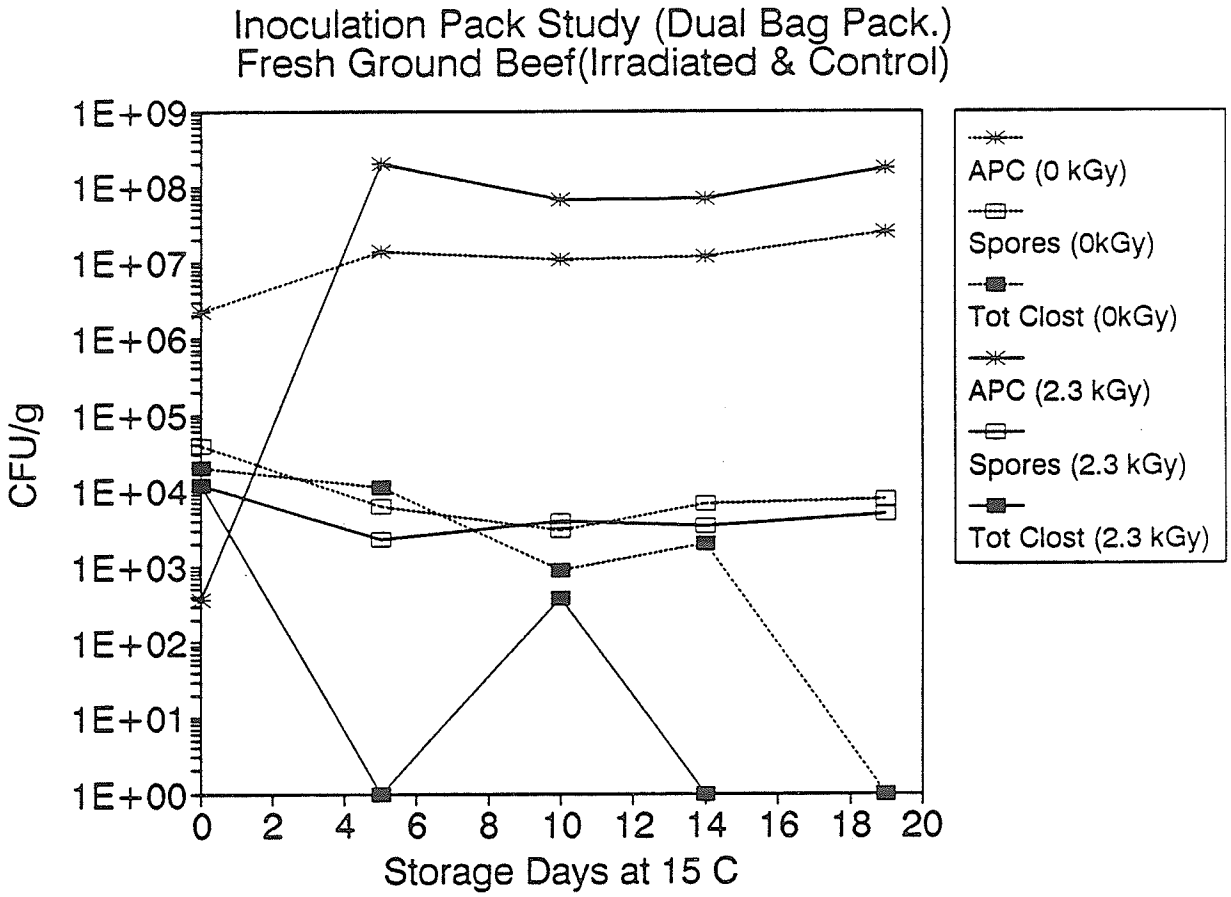
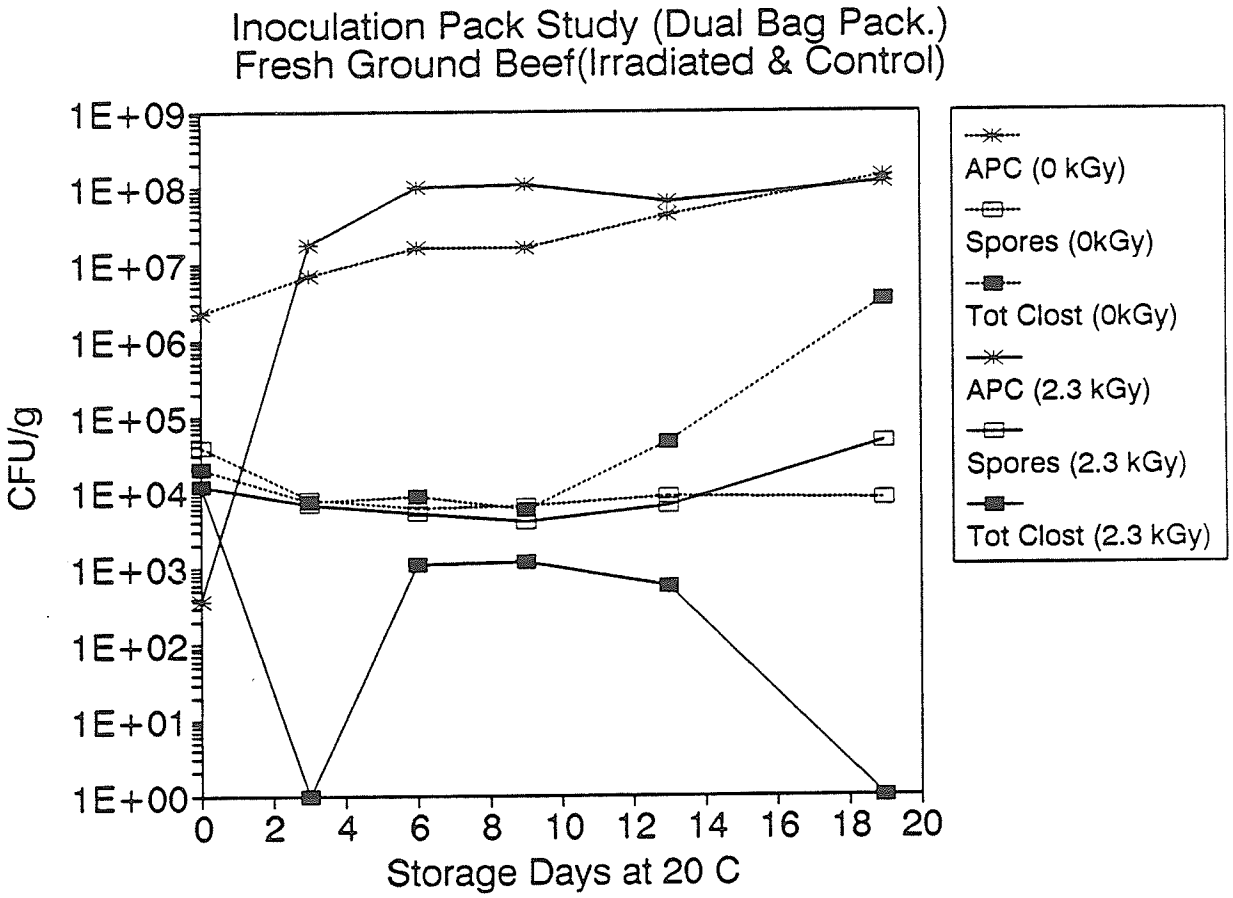

Inoculation Pack Study (Dual Bag Pack.)
Fresh Ground Beef(Irradiated & Control)
CFU/g
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
1E+00
0
2
4
6
8
10
12
14
16
18
20
Storage Days at 15 C
APC (0 kGy)
Spores (0kGy)
Tot Clost (0kGy)
APC (2.3 kGy)
Spores (2.3 kGy)
Tot Clost (2.3 kGy)

Figure 19.


Inoculation Pack Study (Dual Bag Pack.)
Fresh Ground Beef(Irradiated & Control)
CFU/g
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
1E+00
0
2
4
6
8
10
12
14
16
18
20
Storage Days at 20 C
APC (0 kGy)
Spores (0kGy)
Tot Clost (0kGy)
APC (2.3 kGy)
Spores (2.3 kGy)
Tot Clost (2.3 kGy)

The pH of the medium in the petri dishes was measured at three positions on the surface of each plate to give an average pH of the medium in that plate. The SC+3-NH plates from day 10 of storage at 15°C, were the first samples to be evaluated and the results on the pH and CFU are shown in Table 11. The pH of the media used does drop considerably for the irradiated samples.

Table 11. SC+3-NH Media Evaluation for CFU and pH of Dual Bag Packaged Inoculated Patties After Day 10 of Storage at 15°C

Dose (kGy)	Media	Dilution Counted	CFU/g White	Media pH	Dilution Counted	CFU/g Black	Media pH
0	SC+3	10^5	$6.4x10^6$	6.80	10:1	$8.9x10^2$	7.10
2.3	SC+3	10^5	$4.7x10^7$E	5.22	10:1	$3.8x10^2$	5.50

E = Estimates

Three potential solutions to inhibit the pH reduction of the SC+3 medium by the growth of LAB on the medium were initially considered:

1) Addition of antibiotics to produce a more selective medium which would inhibit the growth of lactic acid bacteria (LAB) and thus eliminate the pH reduction resulting for the production of lactic acid produced during the growth of these organisms.

2) Changing incubation temperature from 35°C to inhibit the growth of LAB.

3) Addition of buffers to the SC+3 medium, not to inhibit LAB from growing but to stabilize the pH of the medium when the LAB grow and produce lactic acid on the medium.

The first option was discounted because Vidal and Collins-Thompson (1987) found a wide range of resistance by various lactic acid bacteria to antibiotics. The complexity of the microflora of ground beef along with the unavailability of a general all-purpose antibiotic for all the species potentially contained in ground beef, rendered this option unlikely of success. The second option was ruled out because a reduction in the incubation temperature would not inhibit LAB growth as many are known to grow more effectively at 20°C than at 35°C. An increase in incubation temperature would lead to increased H_2S gas production by C.sporogenes. This gas production causes problems in counting colonies as the medium may either break up or become totally discoloured (black) from the H_2S reacting with the medium.

Including a buffer in the SC+3 medium was investigated as a potential solution to control the pH of the media to LAB growth in irradiated samples. Several concentrations of the phosphate and Tris buffer in the SC+3 medium were investigated. The final buffer concentration in the SC+3 medium was 0.05 Molar. The Health Protection Branch MFA-23 recommends the pH of the SC medium to be 7.6. We compared the recovery of C. sporogenes on the buffering ability of the phosphate and Tris buffers to maintain appropriate pH of the SC+3 media.

The 0.05 Molar phosphate buffered SC+3 medium (SC+3+B5) was prepared as stated in section 5.2.5, modified as follows:

Filter sterilized phosphate and Tris buffered stock solutions were used to prepare the following media:

1) 0.01 M phosphate buffered SC+3 medium (SC+3+B1), pH 7.6.

2) 0.05 M phosphate buffered SC+3 medium (SC+3+B5), pH 7.6.

3) 0.01 M Tris buffered SC+3 medium (SC+3+T1), pH 7.6.

4) 0.05 M Tris buffered SC+3 medium (SC+3+T5), pH 7.6.

The results for inoculated patties stored for 13 days at 20°C were first evaluated with SC+3 medium and SC+3+B1 medium. The results are shown in Table 12. The number of clostridial spores as monitored by the SC+3-H assay were $6.4x10^3$/g and $8.8x10^3$/g for the irradiated and unirradiated control patties, respectively. There were $4.8x10^3$/g spores monitored with the SC+3+B1 medium in irradiated patties, this being fairly close to the number found with SC+3 medium. The pH of the medium for the heat-treated irradiated samples were approximately 7.9 for either medium. This was expected as LAB were not present in the heat-treated assays, thus the pH reduction due to the lactic acid production would not be a problem. The number of total clostridia should be greater than or equal to the number of clostridial spores alone if the total clostridial (non heat-treated) assay is to be effective. As discussed previously, the number of white colonies represents the AnPC of the samples. A portion of these organisms would be LAB. The AnPC of the irradiated and unirradiated control patties were similar at $4.3x10^7$/g and $6.2x10^7$/g, respectively (Table 12). These values were also similar

to the corresponding APC values (Fig. 19). The number of total clostridia monitored in 2.3 kGy irradiated patties with either medium and the pH of that medium was lower than the $6.4x10^3$ spores/g and pH 7.9 monitored with the heat-treated spore assay, respectively. This indicates poor recovery by the total clostridia assay with these samples. The total clostridia monitored as black colonies in the samples and pH of media were lower for SC+3 medium than for SC+3+B1 medium at $5.8x10^2$ CFU/g, pH 5.65 and $3.9x10^3$/g, pH 6.75, respectively. Thus, the addition of phosphate buffer somewhat aided in maintaining the pH closer to the recommended level of 7.6 and thus improved the recovery of the total clostridia, but sufficient recovery was not achieved. The unirradiated control patties showed higher total clostridia than the clostridial spores, as expected, since LAB would not be as dominant a factor in the microflora of these samples as compared to irradiated samples. The total clostridia recovered were ~4.5 and $2.9x10^4$/g for the SC+3 and the SC+3+B1, respectively, which were greater than the 4.8 and $8.8x10^3$ spores/g recovered for the SC+3+B1 and the SC+3 media, respectively. The medium pH of approximately 7.2 was not reduced to the same extent as with the irradiated samples. The pH reduction and effect on improved recovery of total clostridia by the buffered media were not evident with unirradiated control patties, as expected, since LAB would not be as dominant a factor in the microflora of these samples.

Table 12. Evaluation of Various Media for Heat- and Non Heat-Treated Dual Bag Packaged Inoculated Patties Stored at Different Temperatures

Dose (kGy)	Media	Heat Treat	CFU/g White	pH	CFU/g Black	pH
DAY 13 of STORAGE at 20°C						
2.3	SC+3	H			$6.4x10^3$	7.90
2.3	SC+3+B1	H			$4.8x10^3$	7.85
0	SC+3	H			$8.8x10^3$	
2.3	SC+3	NH	$4.3x10^7$	5.35	$5.8x10^2$	5.65
2.3	SC+3+B1	NH			$3.9x10^3$	6.75
0	SC+3	NH	$6.2x10^7$	7.10	$4.5x10^4$	7.20
0	SC+3+B1	NH			$2.9x10^4$	7.25
DAY 14 of STORAGE at 15°C						
2.3	SC+3	H			$3.4x10^3$	
2.3	SC+3+T5	H			$3.2x10^3$	
0	SC+3	H			$6.8x10^3$	
0	SC+3+T5	H			$6.0x10^3$	
2.3	SC+3	NH	$7.4x10^7$		<5	5.5
2.3	SC+3+T5	NH	Sample #1		<5	5.4
			Sample #2		$3.8x10^2$	6.0
0	SC+3	NH	$1.4x10^7$		$2.0x10^3$	7.6
0	SC+3+T5	NH			$1.2x10^3$	7.4

These results all support the idea that the LAB present in the non heat-treated assays are reducing the total clostridia recovery effectiveness due to the pH reduction of the plated medium. The addition of 0.01 M phosphate buffer to

the SC+3 medium was not enough to inhibit this pH reduction. Investigation of higher buffer concentration (0.05 M) in the medium was therefore conducted.

Table 12 also shows results for irradiated and unirradiated patties stored for 14 days at 15°C evaluated using SC+3 and SC+3+T5 media. The number of clostridial spores monitored with the SC+3-H assay in irradiated and unirradiated control patties were $3.4x10^3$/g and $6.8x10^3$/g, respectively. Similar numbers were observed using the SC+3+T5-H assay, indicating the addition of the 0.05 M Tris buffer did not improve the recovery of clostridial spores; it actually showed a slight drop.

The AnPC, monitored as white colonies on the non heat-treated assay showed slightly higher values in irradiated patties than unirradiated control patties, $7.4x10^7$/g vs. $1.4x10^7$/g, respectively. The number of total clostridia was monitored as black colonies on the non heat-treated assays. The number of total clostridia monitored with SC+3 and SC+3+T5 media were less than the number of clostridial spores monitored for corresponding irradiated and unirradiated control patties. This, as before, indicates a recovery problem with the non heat-treated total clostridial assays. Surprisingly, the irradiated patties monitored with SC+3 medium showed no black colonies in this run, and had a medium pH of 5.5. When monitored with SC+3+T5 medium, sample #1 and #2 had 0 and $3.8x10^2$/g with a media pH of 5.4 and 6.0, respectively. We have no simple explanation for this result.

The total clostridia for unirradiated control patties monitored with SC+3 and SC+3+T5 were $2.0x10^3$/g and $1.2x10^3$/g, respectively. The media pH of these assays were 7.6 and 7.4, respectively. The higher pH was anticipated since LAB were not expected to be as major a component of the microflora as was expected with irradiated patties.

The addition of Tris buffer to the SC+3 medium demonstrated limited success in maintaining media pH and improving recovery of total clostridia. The addition of other buffers to maintain pH of media in the non heat- treated total clostridia assay was thus found to be of limited value. The pH reduction of media on non heat-treated assays, especially in the irradiated patties (where LAB were anticipated to be a dominant component of the microflora) was observed. This fact, along with the absence of the pH reduction in heat-treated assays and limited reduction of pH in unirradiated control patties (where LAB were not expected to be a dominant component of the microflora) helped identify LAB as the cause of the pH reductions. The limited success of the buffers in maintaining pH concurrent with slightly improved total clostridia recovery also supported the concept of the pH reductions leading to the reduced recovery efficiency of the assays.

5.2.10.2 Re-evaluation of Stored Samples with Increased Incubation Time

Due to the limited success of 0.01 M phosphate buffered and 0.05 M Tris buffered SC+3 media to monitor total clostridia in irradiated stored samples, further investigation of phosphate and Tris buffered media were conducted using 0.01 M and 0.05 M concentrations for both types of buffer. The investigation was conducted on 2.3 kGy irradiated samples previously evaluated as they demonstrated the highest propensity toward lowering the pH of the assay medium (Table 12). These samples had been stored at 4°C in the 10:1 dilution since their initial evaluation. The 13-day (at 20°C) samples were stored an extra three days at 4°C, while the 14-day (at 15°C) samples were stored an extra two days at 4°C prior to this experiment. The results of this investigation are shown in Table 13. In addition to monitoring the effect of the various buffered media on recovery of total clostridia, the effect of an additional 12 hours of incubation was investigated. The additional incubation time was investigated when faint black colonies were observed with some media. The lack of sufficient incubation time was considered a possible reason for the poor formation of black colonies.

The results for the irradiated patties stored 13 days at 20°C are also shown in Table 13. There were 6.4×10^3 clostridial spores/g monitored with the SC+3-H assay. After 48 hours of incubation no total clostridia were observed with the 0.01 M Tris buffered medium (SC+3+T1) while 4.8×10^3 faint black colonies/g were observed with the 0.05 M buffered medium (SC+3+T5). A further 12 and 24

hours of incubation resulted in the appearance of an estimated $3.6x10^2$ and $2.5x10^3$, respectively, faint black colonies/g with the SC+3+T1 medium. Over the same increase in incubation time the faint black colonies on the SC+3+T5 medium became darker and they were recorded at a level of $2.3x10^3$/g and $3.6x10^3$/g for the two incubation time increases, respectively. The pH of the medium after 72 hours of incubation was 5.9 and 6.6 for the SC+3+T1 and SC+3+T5 medium, respectively. These counts are better but still lower than the spore count.

The 0.05 M phosphate buffered SC+3+B5 media showed better recovery of total clostridia compared to corresponding concentrations of Tris buffered SC+3+T5 media after the standard 48 hour incubation period (Table 13). When incubated 48 hours, the SC+3+B1 and SC+3+B5 media showed $6.4x10^3$ faint black colonies/g and $5.8x10^3$ black colonies/g. After a further 12 and 24 hours of incubation the colonies on the SC+3+B1 became black then faded again while the colonies observed decreased to $1.4x10^3$/g, and further to $9.8x10^2$/g, respectively. Over the same increase in incubation time the colonies on SC+3+B5 medium remained black and essentially unchanged at $7.7x10^3$/g and $3.8x10^3$/g, respectively. The pH of the media after 72 hours of incubation was 7.1 for both SC+3+B1 and SC+3+B5.

These results indicated that the SC+3+B5 was the most effective in recovering total clostridia after the standard 48-hour incubation period; however, after extended incubation the SC+3+T5 medium also showed similar results.

However, there was concern over the lack of development of black coloured colonies in the standard 48-hour incubation period, and the low medium pH with the latter medium.

The results for the irradiated patties stored 14 days at 15°C are shown in Table 13. There were $3.4x10^3$ clostridial spores/g monitored with the SC+3-H assay. After 48 hours of incubation no total clostridia as indicated by black colonies were observed with either the 0.01 M or 0.05 M Tris buffered media assays (SC+3+ T1 or T5). The pH of the media used with either assay was below 5.5. A further 12 hours of incubation resulted in no or limited increases in black colonies observed with even lower pH of the medium. The black colonies observed and media pH for SC+3+T1 and SC+3+T5 media were <5/g (pH 5.2) and an estimated $8.5x10^1$ faint black colonies (pH 5.4), respectively, after 60 hours incubation.

The 0.01 M and 0.05 M phosphate buffered SC+3 media showed much improved recovery of total clostridia compared to corresponding concentrations of Tris buffered media (Table 13). After 48 hours incubation faint black colonies were observed with the SC+3+B1 and SC+3+B5 at $8.9x10^3$/g and $1.2x10^4$/g, respectively. As with the day 13 samples, an additional 12 and 24 hours of incubation generally resulted in fewer colonies which initially became dark followed by fading again. On SC+3+B5 medium an additional 12 hours (total of 60 hours) of incubation did not increase the number or darkness of the colonies, while an additional 24 hours (total of 72 hours) of incubation resulted in fewer

($5.6x10^3$/g) but darker colonies. The pH of the media after 72 hours of incubation was 7.1 for both SC+3+B1 and SC+3+B5.

From these results, we see that phosphate buffered SC+3 media (SC+3+B5) are more effective in recovering total clostridia. Increasing the assay incubation time from 48 to 60 to 72 hours did not increase the number or darkness of total clostridia recovered.

Table 13. Media Recovery Evaluation, Re-Evaluation of Stored Samples, for Black Colony Formation after Different Incubation Times

Inc.	Media							
Time	SC+3+B1	pH	SC+3+B5	pH	SC+3+T1	pH	SC+3+T5	pH
(2.3 kGy, Day 13 at 20°C, 10:1 Dilution Stored 3 Days at 4°C) (6.4×10^3 Spores/g Monitored using SC+3)								
48	$6.4x10^3$#		$5.8x10^3$	7.0	<5	5.3	$4.8x10^3$#	
60	$1.4x10^3$		$7.7x10^3$		$3.6x10^2$#E		$2.3x10^3$	
72	$9.8x10^2$#	7.1	$3.8x10^3$	7.1	$2.5x10^3$#	5.9	$3.6x10^3$	6.6
(2.3 kGy, Day 14 at 15°C, 10:1 Dilution Stored 2 Days at 4°C) ($3.4X10^3$ Spores/g Monitored using SC+3-H)								
48	$8.9x10^3$#		$1.2x10^4$#		<5	5.4	<5	5.5
60	$5.0x10^3$		$1.2x10^4$#		<5	5.2	$8.5x10^1$#E	5.4
72	$7.8x10^2$#	7.1	$5.6x10^3$	7.1	NA	NA	NA	NA

E Estimated
Faint Black colonies

The variability of results observed in different runs with various buffered media resulted in a lack of confidence with even the most effective SC+3+B5

medium. So, a further medium modification was investigated. The exclusion of the dextrose supplement from the base medium resulted in SC+2 medium. This modification was investigated alone and in conjunction with the 0.05 M phosphate buffering. The exclusion of the dextrose limits the carbon source the LAB may utilize to produce lactic acid.

The results of the evaluation of day 19 of storage of irradiated and unirradiated control patties with various media are shown in Table 14. C. sporogenes was monitored with SC+2 and SC+2+B5 in addition to SC+3 and SC+3+B5 media evaluated previously.

Table 14. Evaluation of Various Media, Inoculated Dual Bag Packaged Patties, Irradiated and Control, Stored 19 Days

Dose (kGy)		0	0	2.3	2.3
Temperature (°C)		15°	20°	15°	20°
Patty pH		5.95	6.42	5.28	7.82
Media	Heat Treat.	CFU/g			
APC	No	$2.5x10^7$E	$1.4x10^8$	$1.2x10^8$E	$1.8x10^8$
SC+3	Yes	$7.6x10^3$	$8.0x10^3$	$4.9x10^3$	$4.5x10^4$
SC+3	No	$2x10^2$E	$3.4x10^6$	0	$4.3x10^5$
SC+3	(pH)	(5.5)	(7.3)	(5.3)	(8.0)
SC+3+B5	No	$1.8x10^3$	$3.0x10^3$	$4.5x10^3$	$7.1x10^4$
SC+3+B5	(pH)	(7.4)	(7.4)	(7.2)	(7.6)
SC+2	No	NA	NA	$1.3x10^4$	$1.6x10^5$
SC+2	(pH)	NA	NA	(8.2)	(7.8)
SC+2+B5	No	NA	NA	$6.3x10^3$	$9.3x10^4$
SC+2+B5	(pH)	NA	NA	(8.1)	(8.2)

E Estimated
NA Not Available

The patty pH as measured by the pH of the 1:10 stomached patties were 5.95 and 6.42 for the dual bag packaged unirradiated control patties stored at 15° and 20°C, respectively (Table 14). The number of Clostridial spores as measured by black colonies on the heated SC+3 assay were $7.9x10^3$/g and $8.0x10^3$/g for unirradiated control patties stored at 15° and 20°C, respectively (Table 14). This is similar to the initial inoculation level thus no change has occurred in these samples. The total clostridia as monitored by black colonies on SC+3 medium were an estimated $2x10^2$/g and $3.4x10^6$/g for 15° and 20°C stored samples, respectively. The reduced recovery of C. sporogenes coincides with the reduced pH of the medium (lactic acid bacteria) of 5.5 for 15°C stored samples compared to 7.3 for 20°C stored samples. The higher presence of the LAB in the 15° C stored sample is also indicated by the lower patty pH for these samples (5.95) compared to 20°C stored samples (6.42). The phosphate buffered medium (SC+3+B5) showed similar recovery of total clostridia with $1.8x10^3$/g and $3.0x10^3$/g for 15°C and 20°C stored samples, respectively. The pH of SC+3+B5 medium was 7.4 with both samples, showing the effectiveness of the phosphate buffer in maintaining pH. The recovery of only $3.0x10^3$ black colonies/g with SC+3+B5 medium compared to $3.4x10^6$ black colonies/g with SC+3 medium when monitoring the 20°C stores samples indicates reduced efficiency when phosphate buffer is added to the SC+3 medium.

The pH of patties were 5.28 and 7.82 for the dual bag packaged 2.3 (kGy) irradiated patties stored at 15°C and 20°C, respectively (Table 14). This

indicates, as expected, a greater population of lactic acid bacteria (LAB) in the samples stored at 15°C as compared to 20°C. The clostridial spores monitored with the heat treated SC+3 assay were $4.9x10^3$/g and $4.5x10^4$/g for 15°C and 20°C stored patties. Thus the number of spores only increased above the initial inoculation level of approximately $1x10^4$/g in the 20°C stored samples.

The total clostridia in 15°C stored patties (0/g) could not be monitored effectively by non heat-treated SC+3 medium as expected due to the pH reduction of the medium to 5.3 by the high number of LAB present. The SC+3+B5 medium maintained an acceptable medium pH of 7.2 and showed $4.5x10^3$ total clostridia/g. The total clostridia monitored with the SC medium without the dextrose supplement (SC+2) showed $1.3x10^4$/g while $6.3x10^3$/g were observed with the buffered SC+2+B5 medium. The medium pH using either assay was approximately 8. The most effective medium for monitoring total clostridia in these high LAB populated samples was SC+2.

The total clostridia in 20°C stored patties was $4.3x10^5$/g as monitored by SC+3 medium, with a medium pH of 8.0. This indicates the number of total clostridia has increased from the initial $1x10^4$/g inoculation level. It can be effectively monitored with SC+3 medium as LAB are not a problem as indicated by the 7.82 patty pH. Thus the media pH was not reduced in any of the media tested, which would result in poor recovery of total clostridia. Somewhat reduced recovery was shown with the buffered media, with $7.1x10^4$/g and $9.3x10^4$/g for SC+3+B5 and SC+2+B5 media, respectively. The SC+2 medium

monitored $1.6x10^5$/g with a medium pH of 7.8 which was similar to the results obtained with SC+3 medium.

In samples with a high population of LAB SC+2 medium gave the best recovery of total clostridia. The exclusion of the dextrose carbon source from the supplements reduced the ability of LAB to reduce the pH of the medium by producing lactic acid. Slightly reduced recovery was obtained with either of the buffered media (SC+3+B5 and SC+2+B5) while SC+3 could not monitor total clostridia as the media pH was drastically reduced. In samples without a high LAB component, reduction in media pH and recovery of total clostridia due to media pH reduction was not observed. The recovery of total clostridia with SC+3 or SC+2 media was similar. Thus SC+2 medium was selected to monitor total clostridia inoculated in ground beef. This medium will be tested to see if it can also be used to monitor clostridial spores with the heat-treated assay.

5.2.11 SC+2 Medium Enumeration of Spore Stock

An experiment was conducted to determine if SC+2 medium was effective in monitoring C. sporogenes spores using the heat-treated assay. SC+2 and SC+3 were used to evaluate four samples taken from the combined batch #1-4 of C.sporogenes spores. Each of the four samples was evaluated by duplicate plating with both media types and the average of the duplicates calculated.

The number of spores monitored were: (±1 S.D.)

SC+3: $1.4x10^7$/mL ($\pm 0.1x10^7$)

SC+2: $1.5x10^7$/mL ($\pm 0.1x10^7$)

The acceptability of using SC+2 to monitor clostridial spores using the heat-treated assay was confirmed. The justification of using SC+2 medium to monitor total clostridia (non heat-treated) assay was shown in section 5.2.10.

5.3 Inoculation Pack Studies: Clostridium sporogenes

Clostridium sporogenes spores were inoculated in either radiation sterilized or non sterilized hamburger. The dual packaged patties were subsequently radurized with a dose of approximately 2.5 kGy. Various package storage conditions (dual bag or single bag packaging), and storage temperatures (refrigerated and abuse) were employed as listed in subsequent sections (5.3.1-5.3.4).

Two trials were held to monitor the effect of storage time and temperature on the number of total clostridia (spores and vegetative bacteria) and clostridial spores in radiation (electron Beam) sterilized ground beef (sections 5.3.1 and 5.3.2). A dose of 30 kGy or greater will render ground beef commercially sterile. The inoculation of C. sporogenes spores on a sterilized patty eliminates any effect due to competitive microflora. Surface inoculation was utilized in these experiments to minimize the possibility of undesired recontamination of the sterile patty during inoculation of the spores.

Two trials were held to monitor the effect of storage time and temperature on the number of total clostridia and clostridial spores in fresh ground beef (sections 5.3.3 and 5.3.4). The C. sporogenes spore inoculum was incorporated into the fresh ground beef to give a homogeneous distribution of the inoculum within each patty.

5.3.1 Radiation Sterilized Ground Beef, Trial #1

The initial Inoculation Pack Study with sterilized ground beef involved dual packaged patties stored at 2°, 10°, 15°, and 20°C. The aerobic plate count (APC) and anaerobic plate count (AnPC) of the fresh hamburger on day 0 of storage prior to sterilization was 1.2×10^6 CFU/g and 1.4×10^6 CFU/g, respectively (Table 15). This similarity between APC and AnPC falls within the range found by other researchers for the initial microflora of ground beef. Niemand et Al. (1983) found AnPC values to be approximately 0.1 - 0.2 Log_{10} lower than APC in the ground beef they used, while Lee et Al. (1984) found the AnPC to be 0.9 Log_{10} lower than the APC in their test material. The variation may be explained by AnPC vs. APC results of Madden and Moss (1987) which were 0.6 Log_{10} lower for ground beef made from fresh trim and 1 Log_{10} higher for ground beef made from trim stored vacuum packaged 2 weeks at 1°C prior to grinding. The higher number of anaerobes can be expected if all or a portion of the trim used to make ground beef is stored in vacuum packaging.

The patties were sterilized with an average dose of 48 kGy. Sterilized patties were surface inoculated with C. sporogenes spores after sterilization averaged $2.8x10^4$ total clostridia (vegetative bacteria and spores)/g and $2.3x10^4$ clostridial spores/g (Table 15). An additional dose of 2.3 kGy (electron beam) was given after inoculation to insure similar radurization dose treatment of inoculated spores with subsequent inoculation pack experiments (section 5.3.3 and 5.3.4), and to "sanitize" the patties in the event of accidental contamination during inoculation. This subsequent radiation treatment reduced both the total clostridia and clostridial spores by 0.63 Log_{10} to $1.2x10^4$/g and $1.0x10^4$/g, respectively. The hamburger samples showed no viable aerobic bacteria after irradiations, and there was no growth on storage at any of the storage temperatures.

Uninoculated sterilized control patties did not have any APC, endogenous total clostridia, or clostridial spores on day 0. There were no changes after 11 and 21 days of storage at 20°C, or after 21 and 27 days of storage at 2°, 10°, and 15°C with either dual or single bag packaging, as expected in sterilized ground beef. Thus all total clostridia and clostridial spores present in inoculated patties are due to the inoculum.

Table 15. Inoculation Pack Study, Sterilized Ground Beef, Trial #1 Dual Bag Packaged, Day 0 of Storage

Sterilized	No	Yes	Yes	Yes	Log_{10}
Inoculated	No	No	Yes	Yes	Reduction
Dose (kGy)	0	0	0	2.3	Due to 2.3 kGy
			CFU/g		
APC	$1.2x10^6$	0	0	0	
AnPC	$1.4x10^6$	0	0	0	
Total Clost.	$1.0x10^3$*	0	$2.8x10^4$	$1.2x10^4$	0.33
Clost. Spores	0	0	$2.3x10^4$	$1.0x10^4$	0.36

* Interfering Colonies

5.3.1.1 Storage at 20°C

Patties stored at 20°C showed an increase in total clostridia between day 3 and day 6 of storage (Fig. 20). The clostridial spores did not increase until between day 6 and day 9 of storage. After 11 days of storage the number of total clostridia and clostridial spores were constant at about $4x10^7$/g. Thus all the clostridia at this point were in the spore form. These results were consistent with the increasing number of vegetative clostridia incurring unfavourable environmental conditions such as depletion of available nutrients, or presence of inhibitor substances which could trigger sporulation (Gombas, 1985).

5.3.1.2 Storage at 2°, 10°, and 15°C

The number of total clostridia and clostridial spores in patties stored in dual bag packaging at 2°, 10°, or 15°C for up to 29 days remained the same (Fig. 21). The constant spore and total clostridia levels show there was no germination, and no growth of the inoculated C. sporogenes spores, respectively.

5.3.2 Radiation Sterilized Fresh Ground Beef, Trial #2

The second inoculation pack study with sterilized ground beef involved patties stored in both dual bag and single bag packages at only the abuse temperatures. The storage temperatures monitored were 15°, and 22°-24°C. The day 0 of storage APC and AnPC of the fresh ground beef was $1.1x10^7$ CFU/g and $1.4x10^7$ CFU/g respectively (Table 16). This APC:AnPC ratio falls within the range found by other researchers as noted in section 5.3.1. The initial APC and AnPC values were considered rather high as compared to trial #1 (1 Log_{10} higher), but since this work was done with sterilized hamburger we proceeded with the experiment.

The average sterilization dose delivered to the patties by the E-Beam was 30.8 kGy. In trial #1 a higher sterilization dose (48 kGy) was used; however, a dose of 30 kGy or greater will ensure commercial sterility. Patties were surface inoculated with C. sporogenes spores before the delivered radurization treatment averaged $4.4x10^4$ Total Clostridia/g and $4.6x10^4$ Clostridial Spores/g (Table 16).

The radurization dose (electron irradiation) averaged 2.3 kGy, and reduced both the total clostridia and clostridial spores by 0.53 Log10 to $1.3x10^4$/g and $1.4x10^4$/g, respectively. The inoculated hamburger patties showed no viable APC after sterilization and radurization, and there was no growth on storage at either storage temperature.

Uninoculated sterilized control patties did not have any APC, endogenous total clostridia, or clostridial spores on day 0. There were no changes after 9 and 14 days at 22°-24°C, or 27 days at 15°C with either dual or single bag packaging, as expected from sterilized ground beef. Thus all total clostridia and clostridial spores present in inoculated patties are due to the inoculum.

Table 16. Inoculation Pack Study, Sterilized Ground Beef, Trial #2, Dual Bag Packaged, Day 0 of Storage

Sterilized	No	Yes	Yes	Yes	Log10
Inoculated Reduction	No	No	Yes	Yes	
Dose (kGy)	0	0	0	2.3	Due to 2.3 kGy
	CFU/g				
APC	$1.1x10^7$	0	0	0	
AnPC	$1.4x10^7$	0	0	0	
Total Clost.	$3.6x10^3$*	0	$4.4x10^4$	$1.3x10^4$	0.53
Clost. Spores	0	0	$4.6x10^4$	$1.4x10^4$	0.53

* Interfering Colonies

Figure 20.

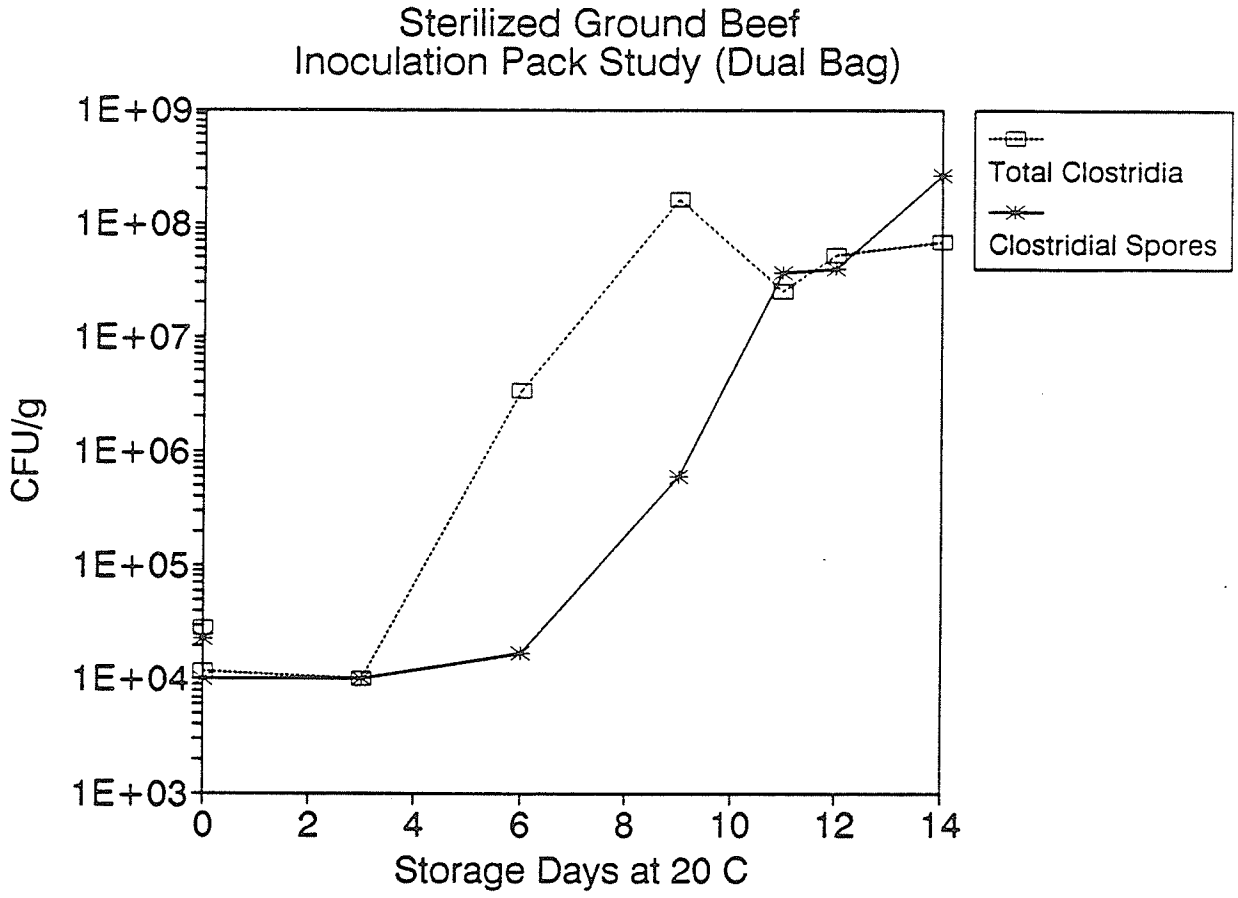
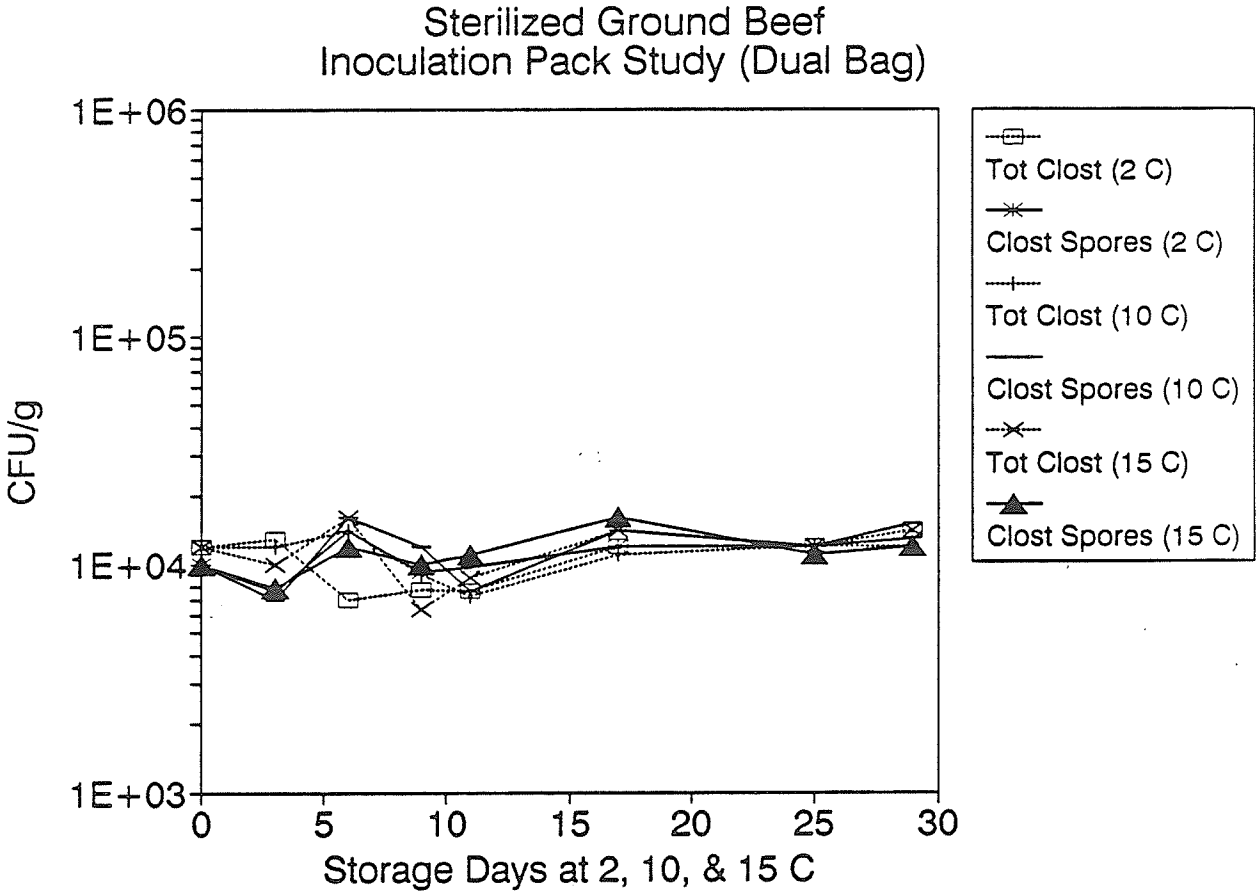

Sterilized Ground Beef
Inoculation Pack Study (Dual Bag)
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
CFU/g
0
2
4
6
8
10
12
14
Storage Days at 20 C
Total Clostridia
Clostridial Spores

Figure 21.


Sterilized Ground Beef
Inoculation Pack Study (Dual Bag)
1E+06
1E+05
1E+04
1E+03
CFU/g
0
5
10
15
20
25
30
Storage Days at 2, 10, & 15 C
Tot Clost (2 C)
Clost Spores (2 C)
Tot Clost (10 C)
Clost Spores (10 C)
Tot Clost (15 C)
Clost Spores (15 C)

5.3.2.1 Storage at 22°-24°C

Inoculated patties stored at 22°-24°C in both the dual and single packages demonstrated similar responses for Total Clostridia and Clostridial Spores (Fig. 22). The number of Total Clostridia increased from the initial $1x10^4$/g to approximately $5x10^6$/g (>2 log increase) between day 3 and 6 of storage. Patties stored in both packaging conditions approached $2x10^8$ Total Clostridia/g by day 12 of storage, although at a slightly slower rate in the single bag packaged samples. The number of Clostridial Spores in patties stored in either packaging condition increased from the initial $1x10^4$/g between days 8 and 9. By day 12 of storage the dual packaged samples approached $1x10^8$/g while the single packaged samples reached $1x10^7$/g.

5.3.2.2 Storage at 15°C

At a 15°C storage temperature the number of total clostridia and clostridial spores in sterilized inoculated patties stored in dual bag and single bag packaging conditions remained constant until day 18 of storage and declined slightly by day 27 (Fig. 23).

Summary

The results of trials #1 and #2 of the inoculation pack studies using radiation sterilized ground beef to eliminate competitive microflora can be summarized as follows:

1) There was no germination or growth of inoculated C. sporogenes spores in patties stored at up to 15°C in either packaging condition (27 and 29 days).

2) Total clostridia growth occurred between day 3 and day 6 of storage in inoculated patties stored at 20°C in dual bag packaging (Trial #1). This was confirmed in trial #2 which showed growth in total clostridia between day 3 and day 6 of storage in patties stored in dual or single bag packages at 22°-24°C. Reintroduction of air into the package (single bag) does not have a significant effect in retarding the C. sporogenes growth in inoculated sterilized patties, abused at room temperature.

5.3.3 Radurized Fresh Ground Beef (Test #1)

In this study the C.sporogenes spore inoculum was homogeneously distributed into fresh ground beef by regrinding the inoculated ground beef 5 times. Ensuring even distribution of the inoculum throughout the patty could be attained as the need for absolute sterility of the patty as required in section 5.3.1 was not required here. The 2.5 kGy (gamma) irradiated dual bag and single bag packaged samples were stored at 15° and 22°C. Samples were monitored for Total Clostridia, Clostridial Spores, APC, AnPC, LAB, and pH.

The initial number of CFU/g assayed for the APC, AnPC, and LAB in the fresh uninoculated commercial ground beef was $1.2x10^6$/g, $1.9x10^5$/g, and $3.3x10^5$/g, respectively. The average initial patty pH was 5.88 (Table 17).

Figure 22.

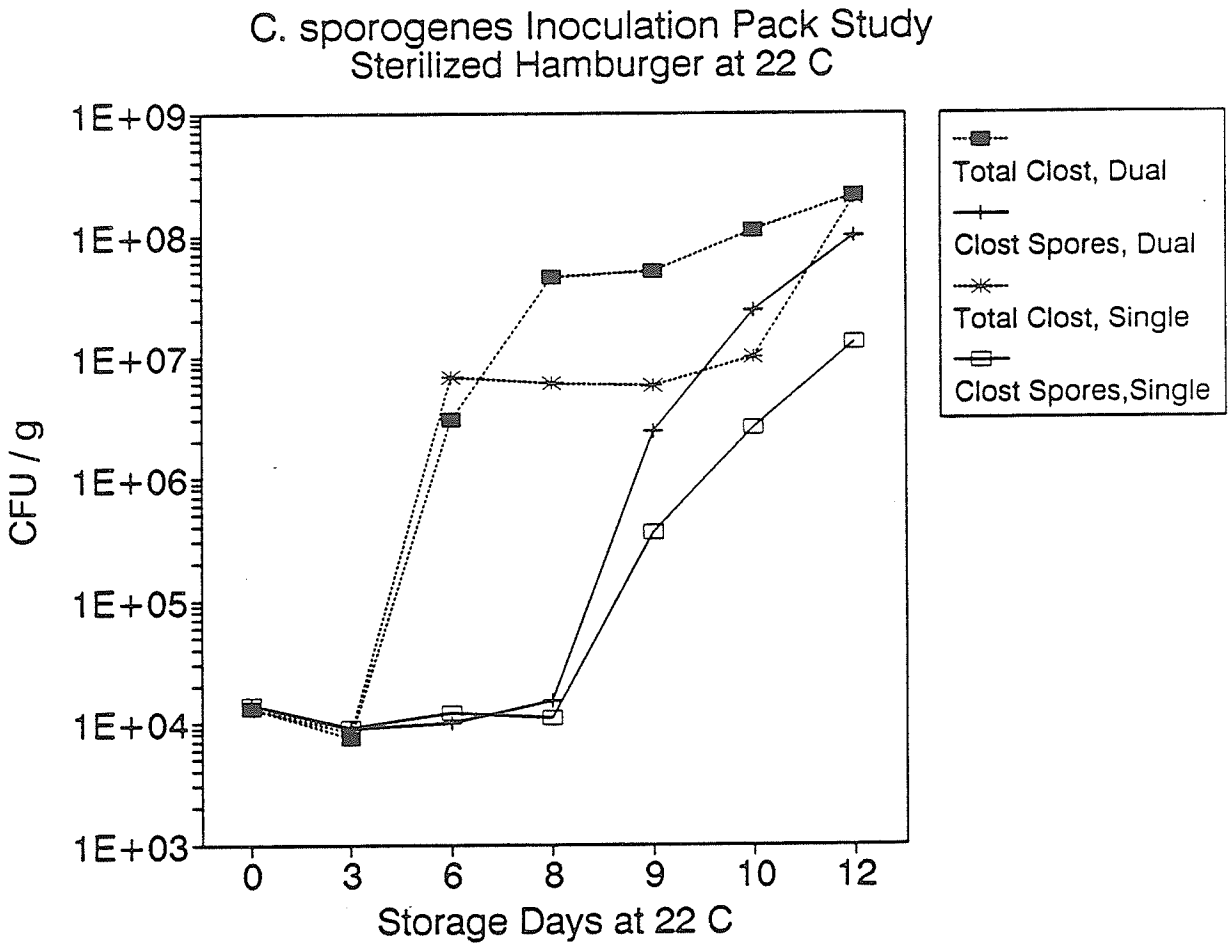
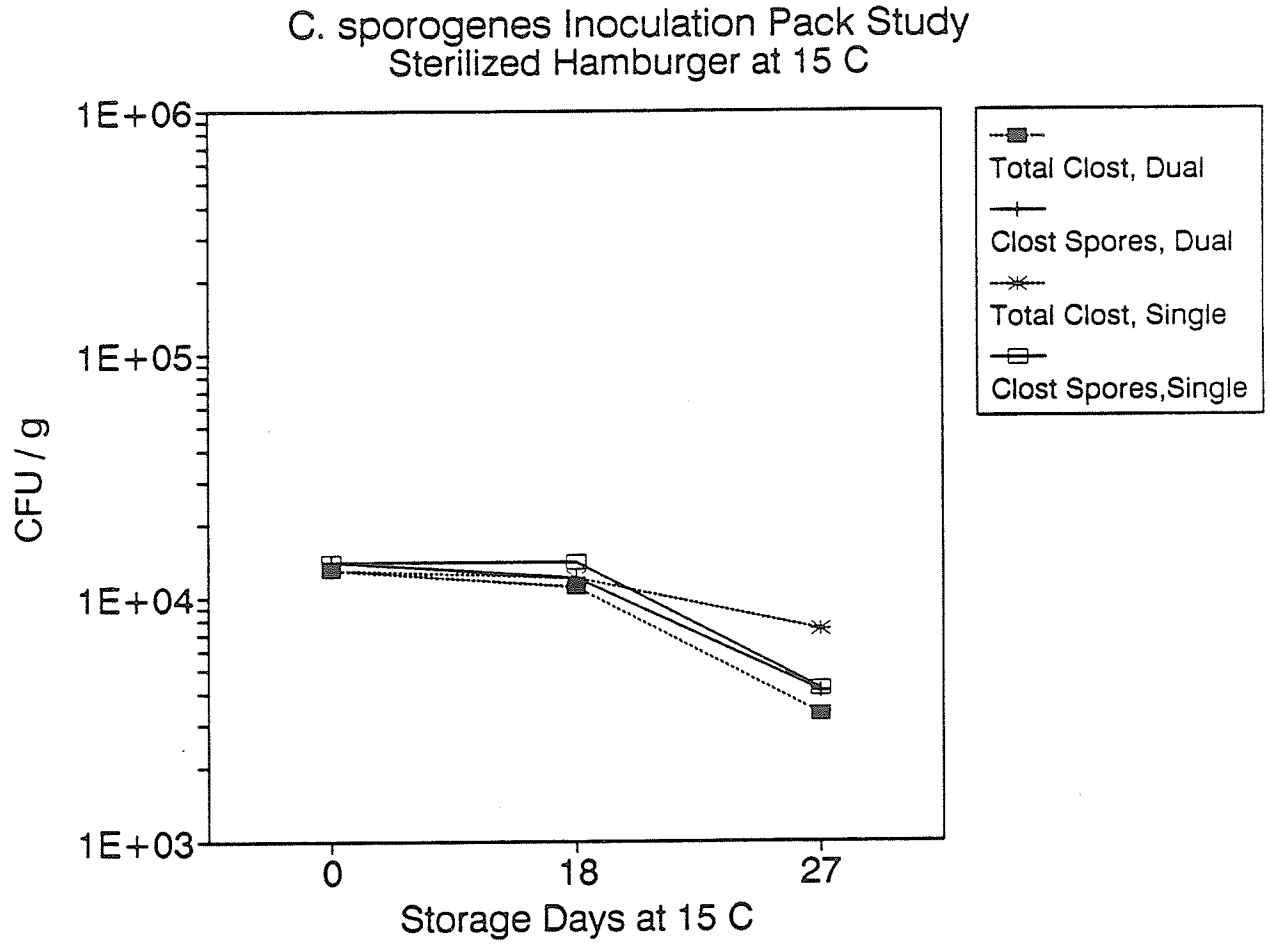

C. sporogenes Inoculation Pack Study
Sterilized Hamburger at 22 C
CFU / g
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
0
3
6
8
9
10
12
Storage Days at 22 C
Total Clost, Dual
Clost Spores, Dual
Total Clost, Single
Clost Spores,Single

Figure 23.


C. sporogenes Inoculation Pack Study
Sterilized Hamburger at 15 C
1E+06
1E+05
1E+04
1E+03
CFU / g
0
18
27
Storage Days at 15 C
Total Clost, Dual
Clost Spores, Dual
Total Clost, Single
Clost Spores,Single

The APC:AnPC ratio falls within the range found by other researchers (Niemand et al., 1983, Lee et al., 1984, Maden and Moss, 1987). There was some interference in the Total Clostridial assay; few non typical tiny black colonies were observed in unirradiated samples. They were estimated to be $2.8x10^2$/g. No Clostridial Spores were observed. There was no interference by tiny black colonies in any of the 2.5 kGy (gamma) irradiated samples. This suggested that these black colonies were destroyed by a 2.5 kGy irradiation dose and thus there was no interference with the results of at least all the irradiated samples.

Initially inoculated samples not subjected to radiation averaged total Clostridia and Clostridial Spores of $5.0x10^4$/g and $2.5x10^4$/g, respectively (Table 17). These inoculated/unirradiated control samples maintained similar values for APC, AnPC, LAB and pH as compared to the uninoculated starting material (Table 17). The average pH for inoculated samples was 5.86. The APC, AnPC, and LAB for inoculated samples averaged $2.2x10^5$/g, $1.3x10^6$/g and $3.4x10^5$/g, respectively, which were very close to the values in the uninoculated samples. This shows that there was no change in the microflora due to the inoculation procedure.

Inoculated samples subjected to 2.5 kGy gamma radiation had the number of Total Clostridia and Clostridial Spores reduced by 0.79 and 0.39 Log_{10}, respectively, to $8.1x10^3$/g and $1.1x10^4$/g (Table 17). The APC declined by about 3 log to $2.8x10^3$ CFU/g and the AnPC and LAB showed similar reductions to $5x10^1$/g (estimated) and $9.8x10^2$/g, respectively (Table 17). Corresponding

uninoculated/irradiated samples possessed similar APC, AnPC, and LAB values as the inoculated irradiated samples, while no total clostridia or clostridial spores were observed in these samples initially or at any time during storage at 15° and 22°C. Thus all total clostridia and clostridial spores monitored were due to the inoculum.

Table 17. Inoculation Pack Study, Fresh Ground Beef Patties Trial #1, Day 0 of Storage

Inoculation	No	Yes	No	Yes	Log10
Dose (kGy)	0	0	2.5	2.5	Reduction
Package	Dual	Dual	Dual	Dual	0-2.5 kGy
pH	5.88	5.86	5.86	5.89	
	----------CFU/g----------				
APC	$1.2x10^6$	$1.3x10^6$	$2.3x10^3$	$2.8x10^3$	
AnPC	$2.8x10^5$	$2.2x10^5$	$8x10^1$E	$5x10^1$E	
LAB	$3.3x10^5$	$3.4x10^5$	$5.2x10^2$	$9.8x10^2$	
Tot. Clost.	$2.8x10^2$*	$5.0x10^4$	0	$8.1x10^3$	0.79
Clost. Spores	0	$2.5x10^4$	0	$1.1x10^4$	0.39

E Estimated
* Tiny Black (Non-typical) C. sporogenes

5.3.3.1 Storage at 22°C

The total clostridia and clostridial spores in inoculated samples stored at 22°C in either packaging condition (dual or single bag) are shown in Fig. 24.

The numbers of total clostridia and clostridial spores up to 6 days of storage were similar and constant, approximately $1x10^4$/g. This illustrates the spore inoculum has not germinated and all total clostridia monitored up to this date are in the spore form. Between days 6 and 8 of storage the number of Total Clostridia and Clostridial spores in the single bag packaged samples increased to $1.1x10^7$/g and $2.2x10^6$/g (estimated), respectively. This suggests some of the inoculated spores in these packages germinated, outgrew, and multiplication of the resultant vegetative sporogenes was followed by sporulation of some of the vegetative sporogenes, within this two-day period, since there is an increase in the number of spores. The same increase in total clostridia and clostridial spores occurred between days 8 and 12 of storage in the dual bag packages. The number of total clostridia and clostridial spores present on day 12 were $6.8x10^7$/g and $2.5x10^7$/g (estimated), respectively.

An extension of allowable storage time at 22°C prior to clostridial growth was seen in radurized (2.5 kGy) patties of inoculated fresh ground beef as opposed to sterilized inoculated patties. Fresh dual and single bag packaged radurized patties showed growth between day 6 and day 8 of storage and between day 8 and day 12 of storage. Sterilized inoculated patties showed growth between day 3 and day 6 of storage in either dual or single bag packaging. These increases in allowable storage of approximately 3 and 5 days for dual and single bag packaged patties appear to be due to the competitive microflora present in the radurized fresh ground beef patties but not present in

the sterilized patties. The microflora present over storage is affected by type of packaging and the storage temperature.

The APC for the dual and single bag packaged samples stored at 22°C were similar, and are shown in Fig. 25. The APC of the irradiated patties increased rapidly from $3x10^3$/g on day 0 of storage to approximately $1x10^6$/g and $1x10^8$/g by day 3 and day 4 of storage, respectively. The APC remained slightly greater than $1x10^8$/g until day 8. The $10^{7.5-8.0}$ ($3.2x10^7$ - $1x10^8$) APC spoilage criteria in ground beef of Jay and Shelf (1978) as modified to be $5x10^7$/g was reached between days 2 and 3. This microbial spoilage level was reached at least 3 days prior to total clostridia growth in single bag packaged patties and 5 days prior to growth in dual bag packaged patties.

A higher percentage of colonies observed on the APC plates from samples at later stages of storage in either packaging condition were typical of yeast and moulds. A yeast and mould (Y&M) assay was included in future studies to monitor this group of organisms.

The APC, AnPC and LAB in dual and single bag packaging inoculated irradiated patties stored at 22°C are shown in Fig. 26. All three groups of organism show similar results in both types of packaging. Concentrations of $1x10^8$/g for all three groups were reached by approximately day 3 of storage and maintained at that level. From these results direct changes in microflora during storage due to the two different packaging conditions cannot be determined, and

thus cannot explain the difference in storage days before growth of total clostridium in the two packaging conditions.

The pH of the patties during storage at 22°C are shown in Fig. 27. Both the inoculated and uninoculated, dual bag package stored patties demonstrated similar pH responses over 8 days of storage. The pH remained constant at about the initial value of approximately 5.9 for the first day of storage, followed by a drop to approximately 5.5 by day 3. There was a gradual increase in pH from this point to approximately pH 5.8 by day 8 of storage. Between day 8 and day 12 of storage, the pH of uninoculated patties remains constant at 5.8, while the inoculated patties' pH increases to 6.4. This may be a result of the increase seen in the total clostridia during this storage period (Fig. 24). The clostridial growth would cause an increase in pH.

The inoculated and uninoculated, single bag packaged patties were also similar in patty pH response over a storage period of 8 days. As with the dual bag packaged samples, the pH remained constant at approximately 5.9 for the first day of storage, followed by a drop to pH 5.5 by day 3 of storage. There was a rapid increase in pH from this point to approximately pH 7 by day 8 of storage (Fig. 27).

The lower pH of dual bag packaged patties may be a contributing factor to the delay in total clostridium growth in dual bag as compared to single bag packaging. The lower pH of patties stored in dual vs. single bag packaging may be a result of differences in microflora, even though a direct difference could not

be seen in the number of colonies (Fig. 26). A greater number of LAB was expected in dual bag packaged patties, to explain the lower pH of patties in this packaging condition; however, this was not seen (Fig. 26). Nassos et al. (1985) did find vacuum packaging resulted in reduced ground beef pH compared to aerobically packaged ground beef, while Jay et al. (1962) found decreased gas permeability of the vacuum packaging resulted in decreased pH.

5.3.3.2 Storage at 15°C

The results of the inoculation pack study with samples stored at 15°C in both packaging conditions are given in Table 18. The APC spoilage level of $5x10^7$/g (Jay and Shelf, 1978) was surpassed at day 15 in both packaging conditions. The APC was $3.0x10^8$/g and $1.7x10^8$/g for dual and single bag packaged samples, respectively. On day 15 there was no increase in total clostridia. These values were $2.0x10^4$/g and $1.3x10^4$/g for dual and single bag packaged samples, respectively, as shown in Table 18. The Clostridial spores present in dual and single bag packaging also remained constant at $5.3x10^3$/g and $9.2x10^3$/g, respectively. No Clostridia were detected in the uninoculated samples. Thus all Clostridia in the inoculated samples were due to the inoculum.

Figure 24.

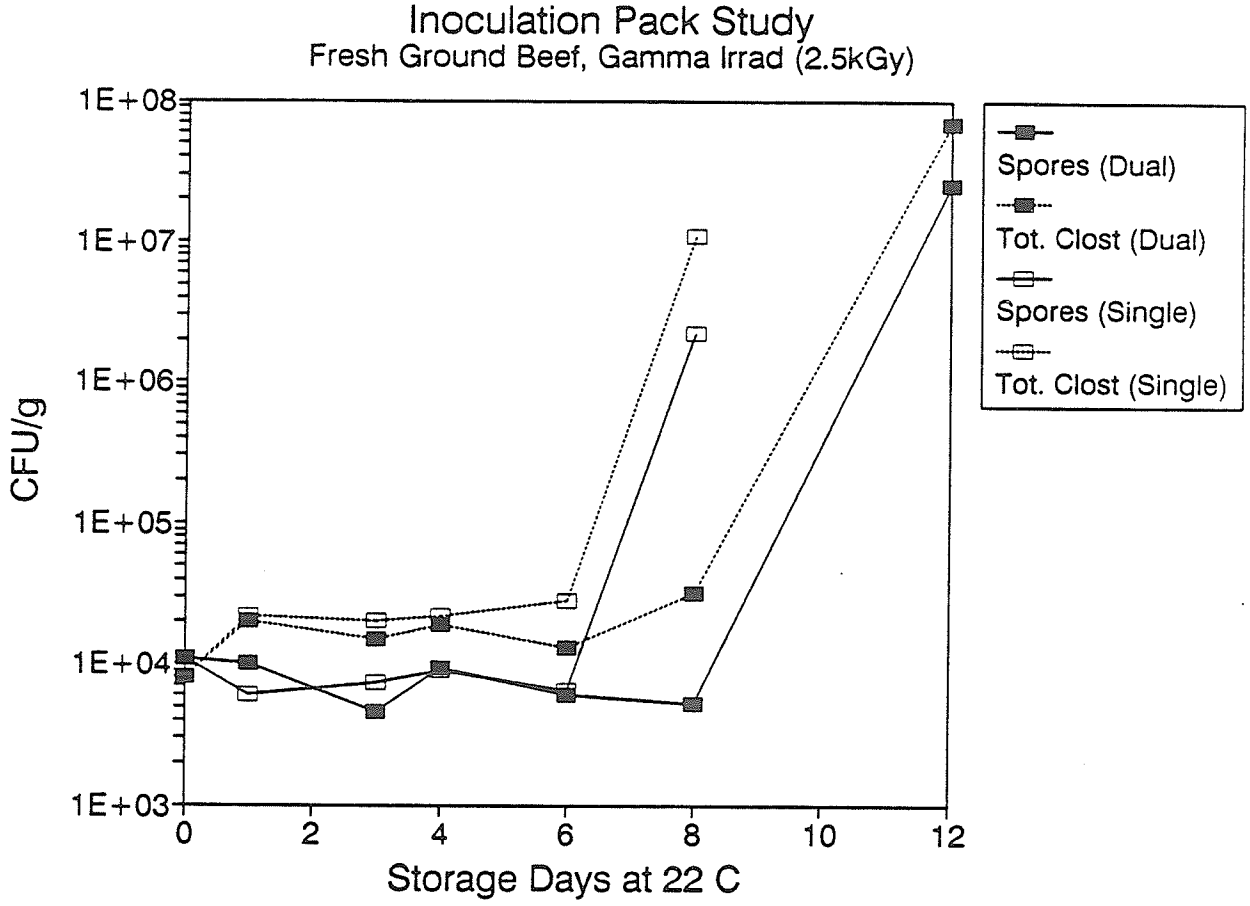

Inoculation Pack Study
Fresh Ground Beef, Gamma Irrad (2.5kGy)
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
CFU/g
0
2
4
6
8
10
12
Storage Days at 22 C
Spores (Dual)
Tot. Clost (Dual)
Spores (Single)
Tot. Clost (Single)

Figure 25.

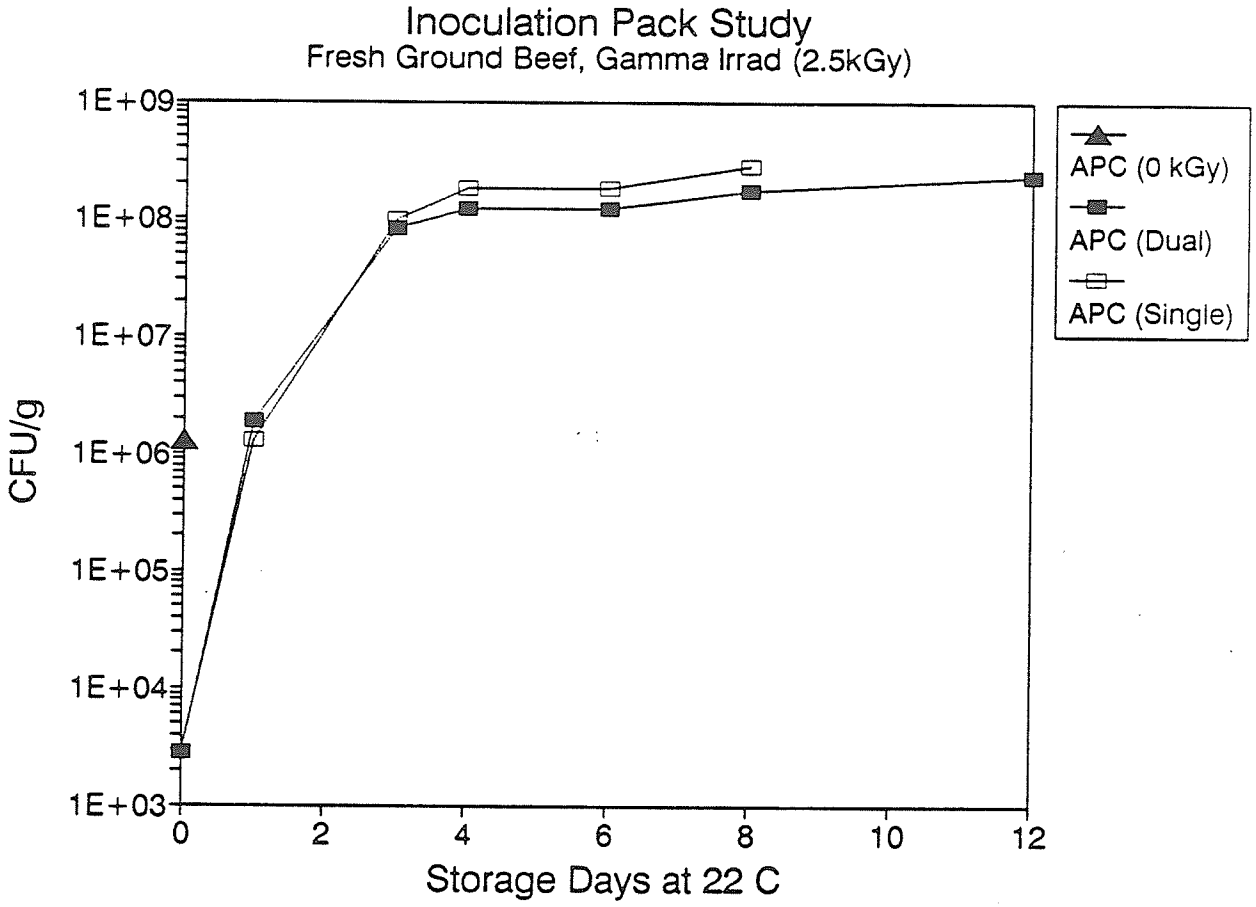

Inoculation Pack Study
Fresh Ground Beef, Gamma Irrad (2.5kGy)
CFU/g
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
0
2
4
6
8
10
12
Storage Days at 22 C
APC (0 kGy)
APC (Dual)
APC (Single)

Figure 26.

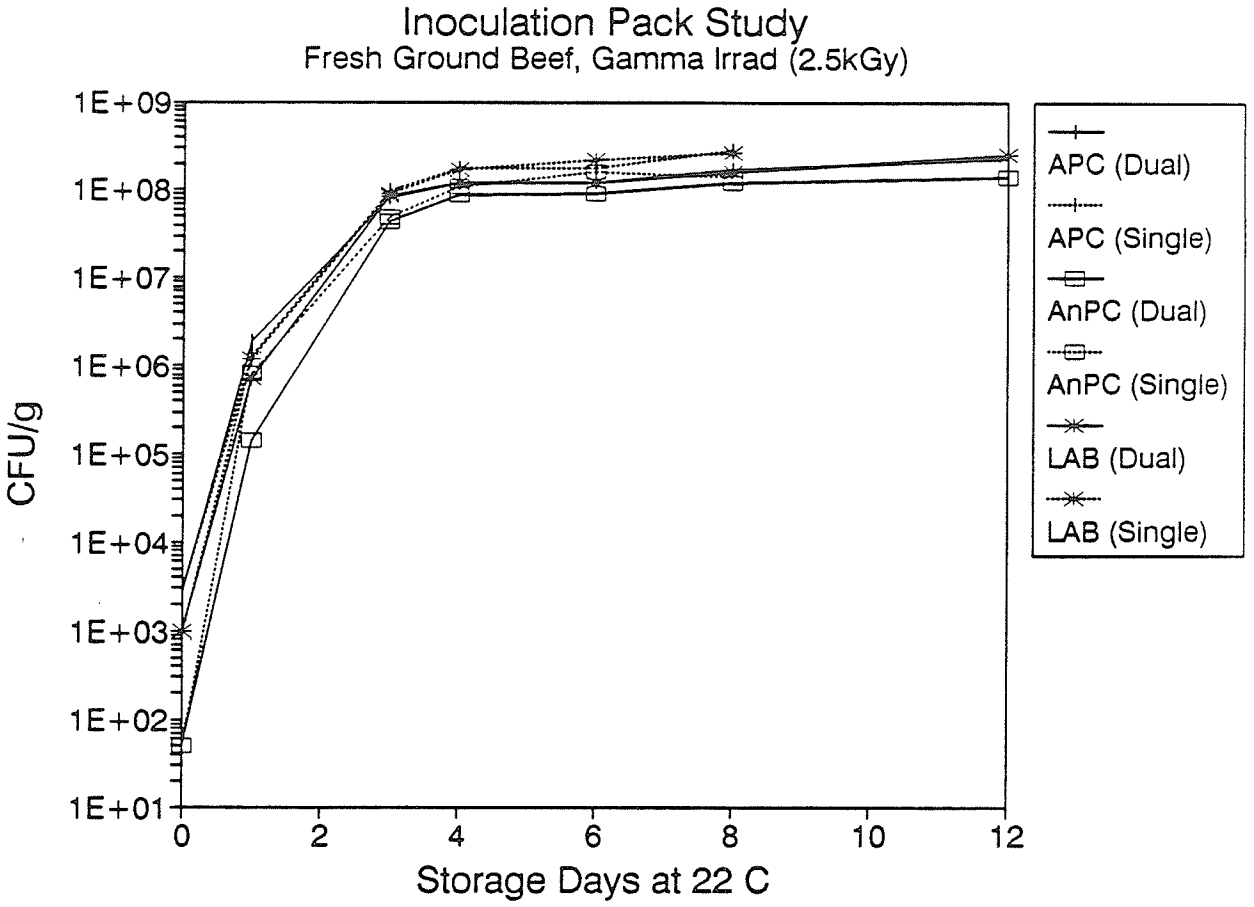
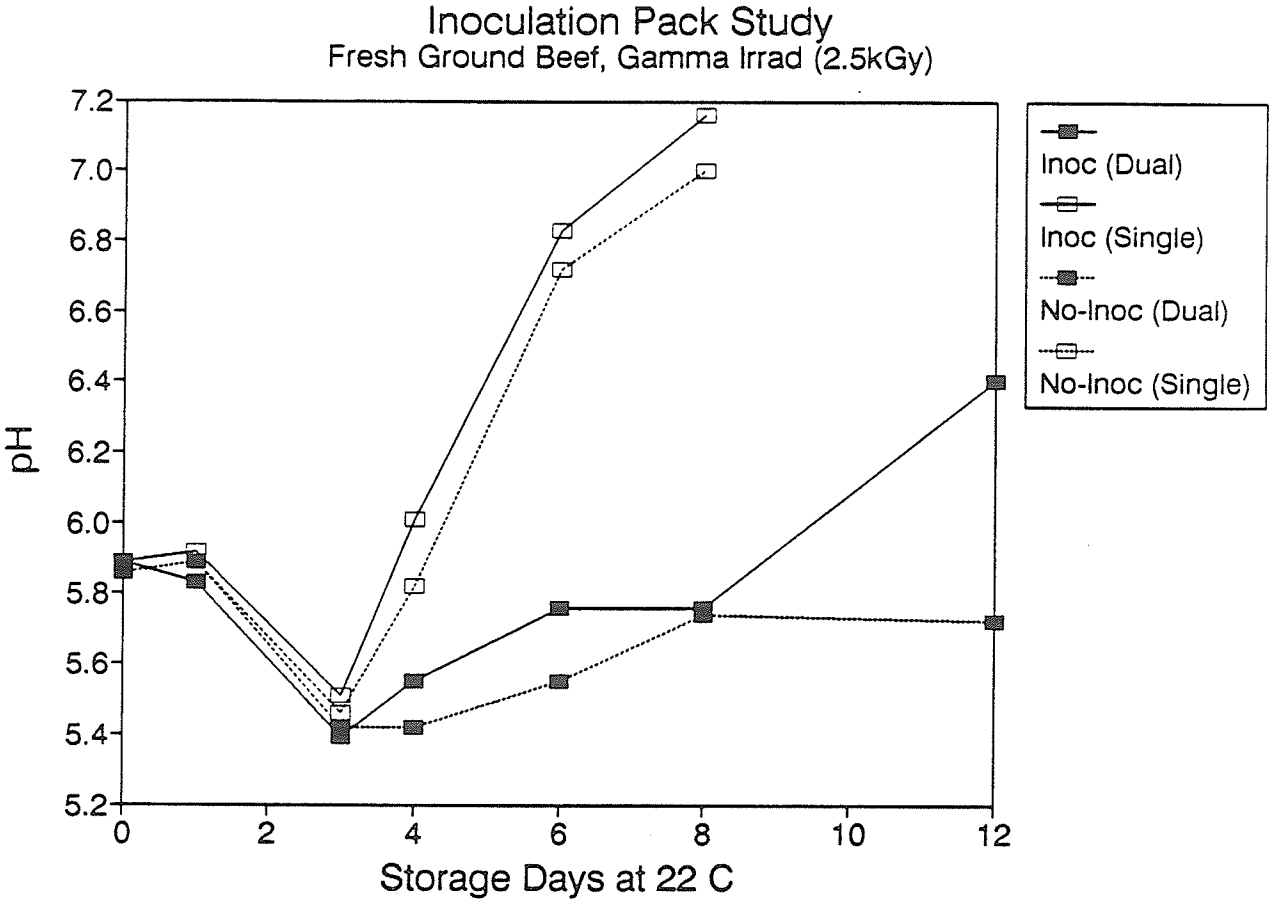

Inoculation Pack Study
Fresh Ground Beef, Gamma Irrad (2.5kGy)
CFU/g
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
0
2
4
6
8
10
12
Storage Days at 22 C
APC (Dual)
APC (Single)
AnPC (Dual)
AnPC (Single)
LAB (Dual)
LAB (Single)

Figure 27.


Inoculation Pack Study
Fresh Ground Beef, Gamma Irrad (2.5kGy)
pH
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
0
2
4
6
8
10
12
Storage Days at 22 C
Inoc (Dual)
Inoc (Single)
No-Inoc (Dual)
No-Inoc (Single)

It should be pointed out there was no contamination problem or change in microflora during inoculation. The APC and pH for uninoculated and corresponding inoculated samples with similar dose treatment and packaging conditions were the same (Table 18).

The pH increased to greater than 7.5 in both inoculated and uninoculated patties irradiated with 2.5 kGy and stored in single bag packaging for 15 days. The corresponding dual bag packaged patties maintained a pH similar to the initial values, with 5.82 and 5.94 for uninoculated and inoculated dual bag packaged patties.

Table 18. Inoculation Pack Study, Fresh Ground Beef Patties Test #1, Initial and Stored at 15°C

Day	Inoc	Dose (kGy)	Pack	pH	Clostridium Sporogenes Total/mL	Clostridium Sporogenes Spores/mL	APC (/g)
0	No	0	Dual	5.88	$2.8x10^{2}$*	0	$1.2x10^{6}$
0	No	2.5	Dual	5.86	0	0	$2.3x10^{3}$
0	Yes	0	Dual	5.86	$5.0x10^{4}$	$2.5x10^{4}$	$1.3x10^{6}$
0	Yes	2.5	Dual	5.89	$8.1x10^{3}$	$1.1x10^{4}$	$2.8x10^{3}$
15	No	2.5	Dual	5.82	0	0	$1.4x10^{8}$
15	No	2.5	Single	7.52	0	0	$2.7x10^{8}$
15	Yes	2.5	Dual	5.94	$2.0x10^{4}$	$9.2x10^{3}$	$1.7x10^{8}$
15	Yes	2.5	Single	7.82	$1.3x10^{4}$	$5.3x10^{3}$	$3.0x10^{8}$

* Tiny black (non-typical C. sporogenes) colonies

5.3.4 Fresh Ground Beef (Test #2)

C. sporogenes spore inoculum was homogeneously distributed into fresh ground beef by regrinding the inoculated ground beef 5 times, and radurized to a dose of 2.5 kGy (electron beam). Radurized and non irradiated control samples were stored at 15° and 22°C in dual bag and single bag packages. Samples were monitored for total Clostridia, clostridial spores, APC, AnPC, LAB, Y&M, and pH.

Table 19. Inoculation Pack Study, Fresh Ground Beef Test #2, Day 0 of Storage

Inoc	Dose (kGy)	Pack	pH	APC/g	LAB/g	Y&M/g
No	0	Dual	5.92	$4.4x10^6$	$2.5x10^6$	$2.1x10^5$
No	0	Single	5.90	$3.8x10^6$	$3.6x10^6$	$1.6x10^5$
No	2.5	Dual	5.91	$4.8x10^3$	$1.6x10^3$	$2.6x10^4$
No	2.5	Single	5.93	$2.5x10^3$	$1.8x10^3$	$2.8x10^4$
Yes	0	Dual	5.91	$4.2x10^6$	$4.0x10^6$	na.
Yes	0	Single	5.91	$3.7x10^6$	$4.2x10^6$	na.
Yes	2.5	Dual	5.96	$2.2x10^3$	$2.9x10^3$	na.
Yes	2.5	Single	5.94	$1.9x10^3$	$1.6x10^3$	na.

The average (dual and single bag) initial APC, LAB, and Y&M assayed in fresh uninoculated ground beef prior to being subjected to radurization was approximately $4x10^6$/g, $3x10^6$/g and $2x10^5$/g, respectively (Table 19). The average initial pH was about 5.9. There was interference in the total clostridia assay and some non typical tiny black colonies were observed. In dual and single bag

packages at day 0 of storage there were an estimated $8x10^1$/g and $2.4x10^3$/g, respectively of these interfering colonies (Table 20). There were no clostridial spores present in either packaging condition at this time.

The average (dual and single bag) initial APC, LAB, and patty pH in fresh inoculated unirradiated control ground beef were maintained at levels similar to the uninoculated patties. They were $4x10^6$/g, $4x10^6$/g, and 5.91, respectively (Table 19). This shows there was no contamination or change in microflora of the patties due to inoculation of spores. The initial total clostridia in dual and single bag packaging on day 0 of storage were $1.2x10^5$/g and $9.0x10^4$/g, respectively (Table 20). The clostridial spores present were $3.7x10^4$/g and $2.0x10^4$/g for dual and single bag packaging, respectively. The difference between total clostridia and clostridial spores was greater than experienced in test #1 on day 0.

Table 20. Inoculation Pack Study, Fresh Ground Beef Test #2, Spore Counts, Day 0 of Storage

Day	Inoc	Dose (kGy)	Pack	pH	Clostridium Sporogenes Total/mL	Clostridium Sporogenes Spores/mL	APC (/g)
0	No	0	Dual	5.92	$8x10^1$E*	0	$4.4x10^6$
0	No	0	Single	5.90	$2.4x10^3$*	0	$3.8x10^6$
0	No	2.5	Dual	5.91	0	0	$4.8x10^3$
0	No	2.5	Single	5.93	0	0	$2.5x10^3$
0	Yes	0	Dual	5.91	$1.2x10^5$	$3.7x10^4$	$4.2x10^6$
0	Yes	0	Single	5.91	$9.0x10^4$	$2.0x10^4$	$3.7x10^6$
0	Yes	2.5	Dual	5.96	$1.2x10^4$	$1.8x10^4$	$2.2x10^3$
0	Yes	2.5	Single	5.94	$1.2x10^4$	$1.2x10^4$	$1.9x10^3$

E Estimated Count
* Tiny black (non-typical C. sporogenes) colonies

Patties subjected to a 2.5 kGy (electron beam) irradiation showed reductions in all groups of organisms monitored as compared to corresponding unirradiated control samples. Uninoculated samples (dual and single bag) had Log10 reductions of 3.1, 3.2 and 0.8 in APC, LAB, and Y&M, to an average of $4x10^3$/g, $2x10^3$/g, and $3x10^4$/g, respectively (Table 19). The inoculated samples showed similar reductions in APC and LAB. A 3.3 Log10 reduction was found for both APC and LAB to an average of $2x10^3$/g in both packaging conditions.

There was no interference from the tiny black colonies in the total clostridia assay for the 2.5 kGy irradiated samples, on day 0 or any day of storage. This suggests they were destroyed by this dose and did not affect any of the irradiated samples. The uninoculated-irradiated patties had no total clostridia or clostridial spores on day 0 or any day of storage at 15°C (Table 21) or 22°C (Fig. 28). The inoculated patties subjected to 2.5 kGy electron beam irradiation had the number of total clostridia reduced by 1.0 and 0.9 $\log_{10}$ for dual and single bag packaging, respectively, from $1.2x10^5$/g and $9.0x10^4$/g to $1.2x10^4$/g for both (Table 21). The clostridial spores were reduced by 0.3 and 0.2 $\log_{10}$ for dual and single bag packaging to $1.8x10^4$/g and $1.2x10^4$/g, respectively.

5.3.4.1 Storage at 22°C

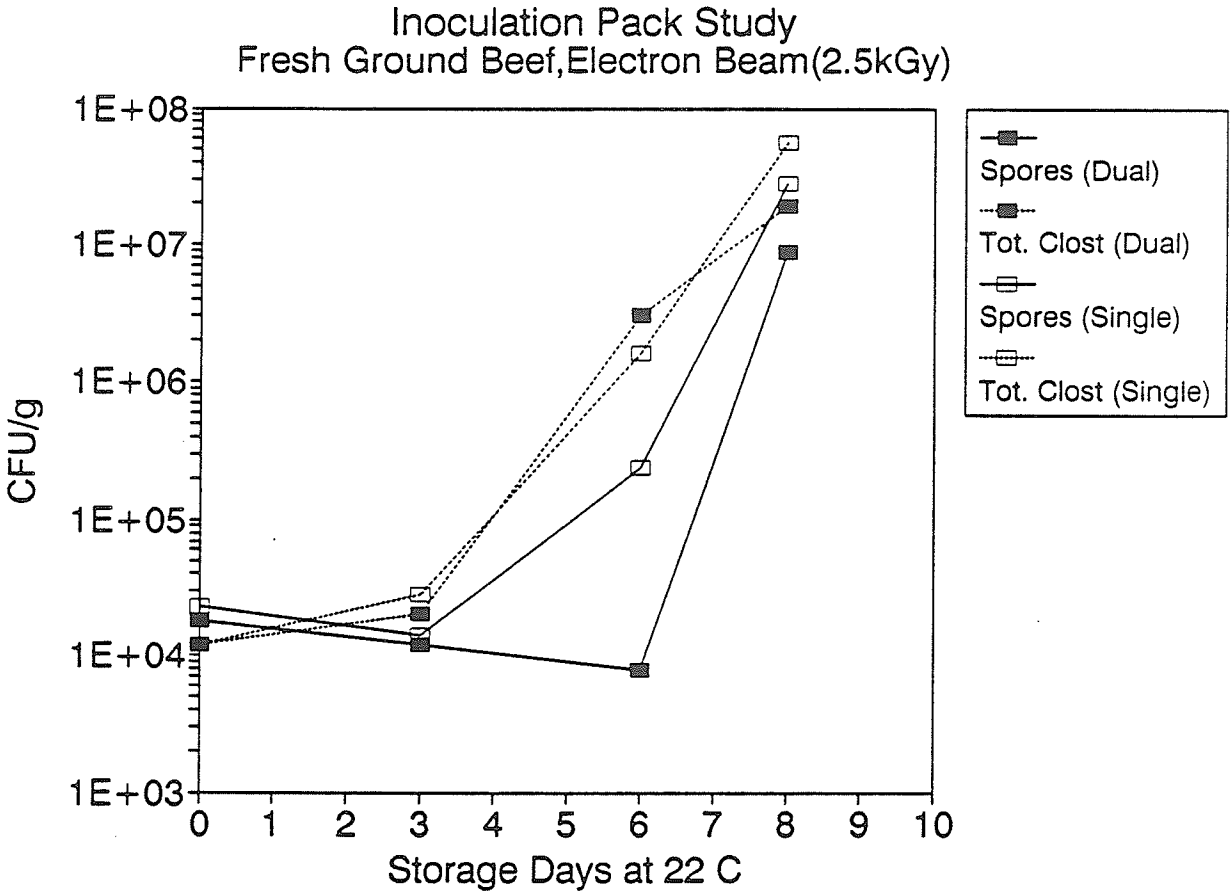
(2.5 kGy Irradiated): The total clostridia and clostridial spores in inoculated patties, electron beam irradiated with 2.5 kGy and stored at 22°C in both packaging conditions (dual and single bag), are shown in Fig. 28. The

number of total clostridia and clostridial spores up to 3 days of storage were similar and remained constant, approximately $1x10^4$/g. This illustrates the spore inoculum did not germinate, and all the total clostridia monitored are in the spore form up to this date. Between day 3 and day 6 the number of total clostridia in both packaging conditions increased to greater than $1x10^6$/g. By day 8 the total clostridia with both packaging conditions was greater than $1x10^7$/g.

There was no appreciable drop in the number of clostridial spores in patties stored in either packaging condition (Fig. 28). This suggests in both packaging conditions, only a few inoculated spores germinated, outgrew and initiated multiplication between day 3 and day 6 of storage. If a significant drop in the number of clostridial spores had occurred in conjunction with an increase in the number of total clostridia, this would have indicated most of the inoculated spores had germinated and outgrown.

The number of clostridial spores in dual packaged samples remained at the $1x10^4$/g level until day 6; however, there was a rapid increase to greater than $1x10^7$/g between day 6 and day 8. The number of clostridial spores in single bag packaged samples increased to greater than $1x10^5$/g between day 3 and day 6. There was a further increase to greater than $1x10^7$/g by day 8. These results suggest the vegetative sporogenes resultant from growth of the inoculum reverted back into the spore form by sporulation between day 6 and 8 in dual bag packaged samples, and between day 3 and 6 in single bag packaged samples. The uninoculated samples stored in both packaging conditions had no total clostridia or clostridial spores on day 0 and day 10.

Figure 28.


Inoculation Pack Study
Fresh Ground Beef,Electron Beam(2.5kGy)
CFU/g
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
0
1
2
3
4
5
6
7
8
9
10
Storage Days at 22 C
Spores (Dual)
Tot. Clost (Dual)
Spores (Single)
Tot. Clost (Single)

The APC for irradiated samples in both packaging conditions increased from approximately $2x10^3$/g initially to $1x10^8$/g by day 3 (Fig. 29). Thus the $5x10^7$/g APC spoilage criterion of Jay and Shelf (1978), was surpassed prior to day 3 of storage. This detectable spoilage was reached prior to growth of clostridia, as total clostridia did not increase until between day 3 and day 6 of storage. The APC remained constant at about $1x10^8$/g between day 3 and day 8. The LAB for these samples were similar to the APC (Fig. 29), suggesting the majority of the organisms monitored with the APC are lactics. The Y&M count was also approximately $1x10^8$/g between day 3 and day 6; however, it dropped to $1x10^6$/g by day 8. There were no differences between dual or single bag packaging in APC, LAB, or Y&M counts present in samples stored at 22°C. The results for APC and LAB of patties in both packaging conditions were the same as the results observed in test #1 section 5.3.2.1 (Fig. 26).

The detectable spoilage level was reached prior to day 3 for patties in either packaging condition. Growth of inoculated clostridia in patties stored in either packaging condition did not occur until between day 3 and day 6 of storage. Due to the 3-day time interval between evaluation days, the actual number of days between microbiological spoilage and clostridial growth is unclear. Depending upon at what point between day 3 and day 6 of storage the clostridial growth occurred, there could be as little as 1 day and as many as 4 days of microbiological spoilage prior to clostridial growth. This compares to 5-9

days and 3-5 days of microbiological spoilage prior to growth of clostridia in dual and single bag packaging found in Test #1 (section 5.3.2.1).

The pH of irradiated and unirradiated inoculated patties stored at 22°C are shown in Fig. 30. The initial pH of the patties is about 5.9. The pH of the single bag packaged patties increase more rapidly than the corresponding dual bag packaged patties. By day 6 of storage the patty pH of dual bag packaged irradiated and unirradiated samples increased to approximately 6.7 and 6.1, respectively, while corresponding single bag packaged samples increased much higher to 8 and 7.2, respectively. The pH of unirradiated control samples increased more rapidly than corresponding irradiated samples similarly packaged. There was no initial decrease in patty pH as seen in test #1 (Fig. 27). By day 6 of storage the pH of inoculated, irradiated dual and single bag packaged patties were 5.7 and 6.8 in test #1 as compared to the higher values of 6.1 and 7.2 in test #2.

There was a reduction in the number of days of microbiological spoilage prior to growth of clostridia in patties stored in dual and single bag packaging found in test #2 as compared to test #1 (section 5.3.3.1). The higher pH of the irradiated patties during storage in test #2 compared to test #1 may be the reason for the more rapid clostridial growth. Enfors and Molin (1978) found a pH decrease from 6.7 to 6.0 dramatically reduced the germination of C. sporogenes in a complex media. It was surprising that the LAB count was similar between the two tests, as it was expected that a difference in patty pH

would be a result of a difference in LAB levels. Perhaps our LAB assay was not specific enough to identify the actual number of lactic acid producers. A lack of specificity would explain why the APC and LAB values were similar in test #1 (Fig. 26) and test #2 (Fig. 29).

(Unirradiated Control)

The total clostridia and clostridial spores in unirradiated-control, inoculated patties stored at 22°C in both packaging conditions (dual and single bag) are shown in Fig. 31. The number of total clostridia in both packaging conditions increased from approximately $1x10^5$/g on day 0 to greater than $1x10^7$/g by day 3 of storage. This increase continued, and by day 6 stored patties had greater than $1x10^8$/g, and $1x10^9$/g, in dual and single bag packaged samples, respectively.

The results for total clostridia and clostridial spores monitored in uninoculated samples stored in both packaging conditions are shown in Fig. 32. Unlike the 2.5 kGy treated samples in this test, test #1 and previous inoculation pack studies with sterilized ground beef (section 5.3.1), there were a substantial number of colonies with the total clostridia and clostridial spore assays for the uninoculated 0 kGy treated samples. The number of colonies in the total clostridia assay for samples in either packaging conditions resulted in similarly shaped growth curves. The single bag packaged samples increased rapidly from approximately $2x10^3$/g on day 0 to $5x10^7$/g on day 3, followed by a moderate

increase to $2x10^9$/g by day 6 (Fig. 32). The dual bag packaged samples had a similar growth pattern, with values being approximately 1 log10 lower than corresponding single bag packaged samples. The high values observed indicate there is an organism(s) interfering with the total clostridial assay. The size of the colonies could not be distinguished on the assay plates, due to the large number of colonies per plate, with the dilutions plated.

In both packaging conditions the number of clostridial spores remained constant at approximately $3x10^4$/g up to day 3 of storage in inoculated patties (Fig. 31). Between day 3 and day 6 of storage there was an increase to approximately $1x10^7$/g. Between day 6 and day 8 the number of spores remained about $1x10^7$/g, generally unchanged. During this time the number of clostridial spores showed a slight decrease (dual bag) and slight increase (single bag), respectively.

Previously we saw the number of clostridial spores in inoculated 2.5 kGy irradiated patties did not increase until between day 6 and day 8 of storage in dual bag packaging, while in single bag packaged patties the increase was between day 3 and day 6 (Fig. 28).

The number of colonies observed with the clostridial spore assay for uninoculated samples in either packaging conditions resulted in similarly shaped growth curves (Fig. 32). There were no detectable colonies up to day 3 of storage. On day 6 there were an estimated $4x10^1$/g, and $1.6x10^3$/g for dual and single bag packaged samples, respectively. On day 6 there were an estimated

$2.4x10^5$/g for samples stored in either packaging condition. In previous inoculation pack experiments where samples were treated with 2.5 kGy, no colonies had been observed with the clostridial spore assay.

The degree of interference from background microflora on the total clostridia and clostridial spore assays of the inoculated samples was evaluated by comparing the inoculated and uninoculated sample growth curves. The growth curve of colonies monitored with the total clostridia and clostridial spore assays in inoculated and uninoculated, dual bag packaged samples (Fig. 33) and single bag packaged samples (Fig. 34) are shown. The total clostridia monitored in dual bag packaged samples on day 0 was approximately $1x10^5$/g and $1x10^2$/g, for inoculated and uninoculated samples, respectively (Fig. 33). The total clostridia monitored in single bag packaged samples on day 0 was approximately $1x10^5$/g and $2x10^3$/g, for inoculated and uninoculated samples, respectively (Fig. 34). The total clostridia in inoculated samples are 3 $\log_{10}$ and 2 $\log_{10}$ greater than uninoculated samples, in dual and single bag packaging, respectively, on day 0. Thus there is no mistaking the contribution of the inoculum. The number of colonies monitored with the total clostridia assay was approximately the same for inoculated and uninoculated samples on day 3 and day 6 in both packaging conditions. The high number of colonies in uninoculated samples makes it impossible to determine the contribution of the inoculum in unirradiated control patties after day 0 of storage (Figs. 33 and 34).

Figure 29.

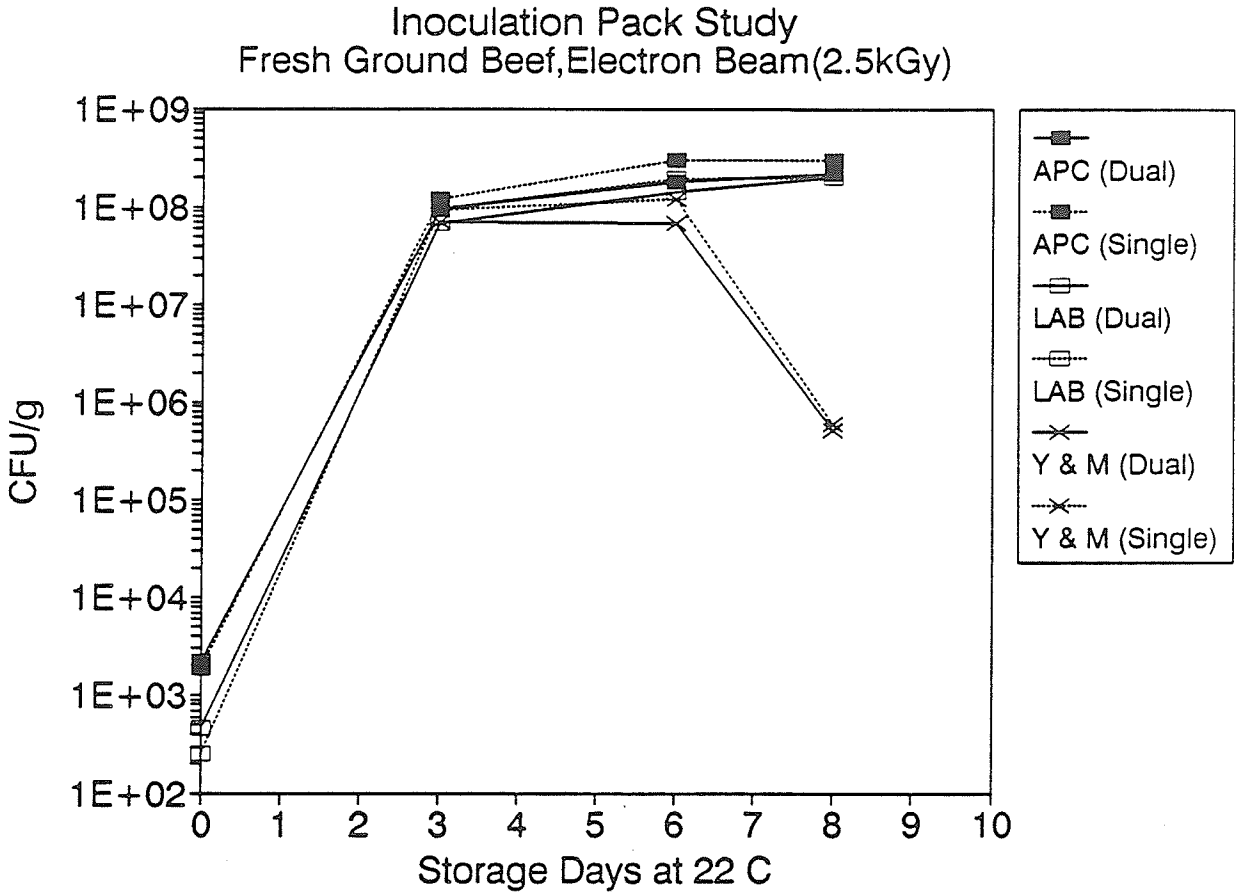

Inoculation Pack Study
Fresh Ground Beef,Electron Beam(2.5kGy)
CFU/g
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
0
1
2
3
4
5
6
7
8
9
10
Storage Days at 22 C
APC (Dual)
APC (Single)
LAB (Dual)
LAB (Single)
Y & M (Dual)
Y & M (Single)

Figure 30.

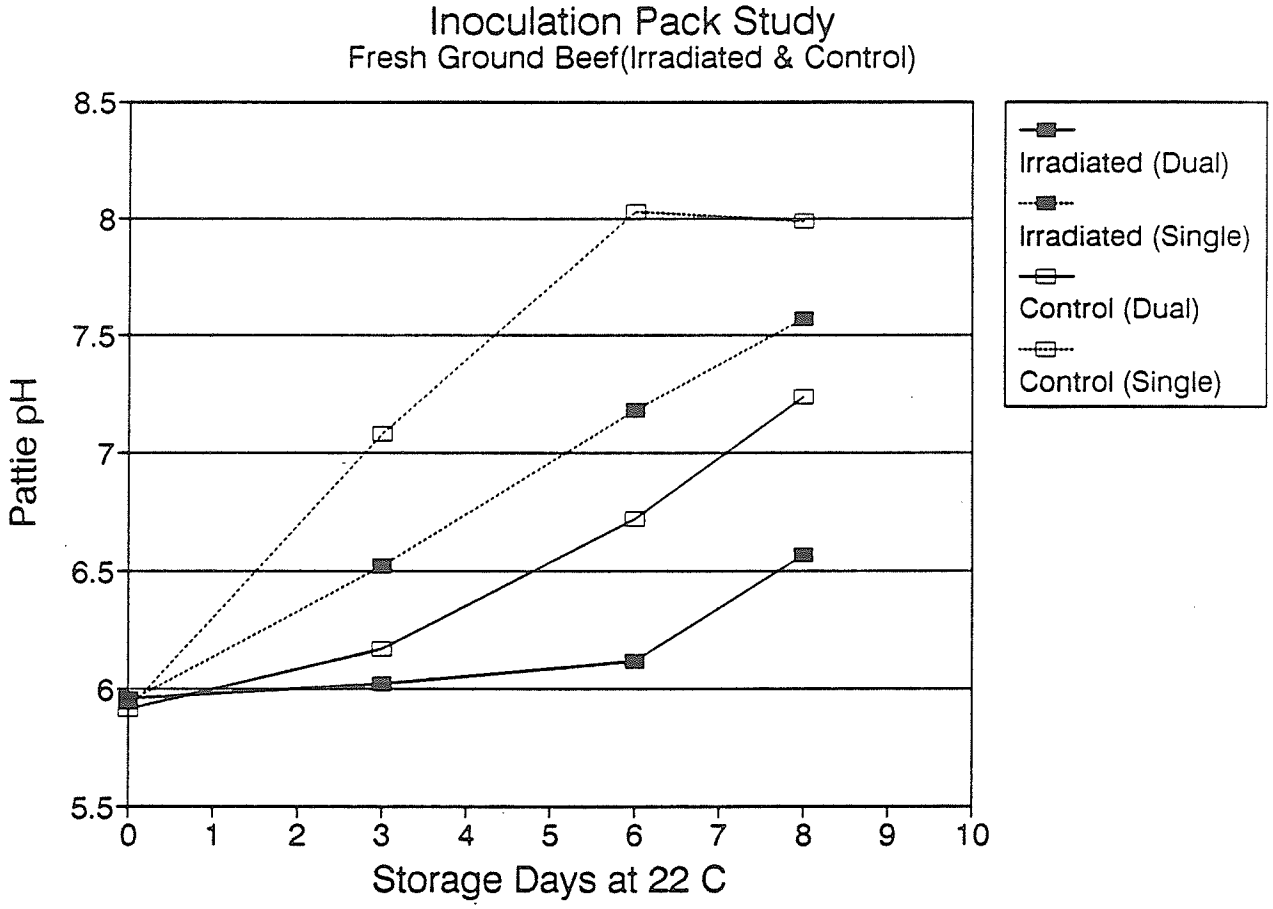

Inoculation Pack Study
Fresh Ground Beef(Irradiated & Control)
Pattie pH
8.5
8
7.5
7
6.5
6
5.5
0
1
2
3
4
5
6
7
8
9
10
Storage Days at 22 C
Irradiated (Dual)
Irradiated (Single)
Control (Dual)
Control (Single)

Figure 31.

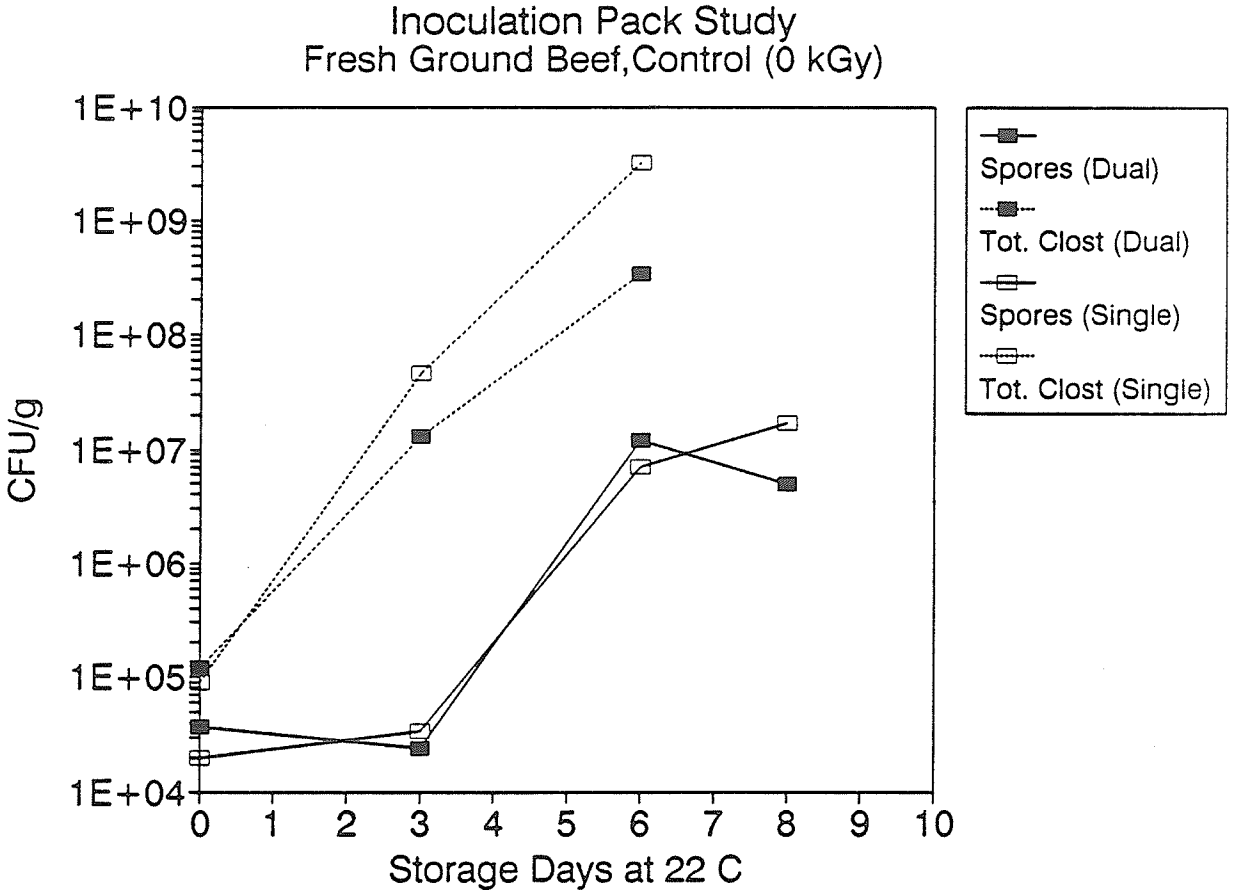
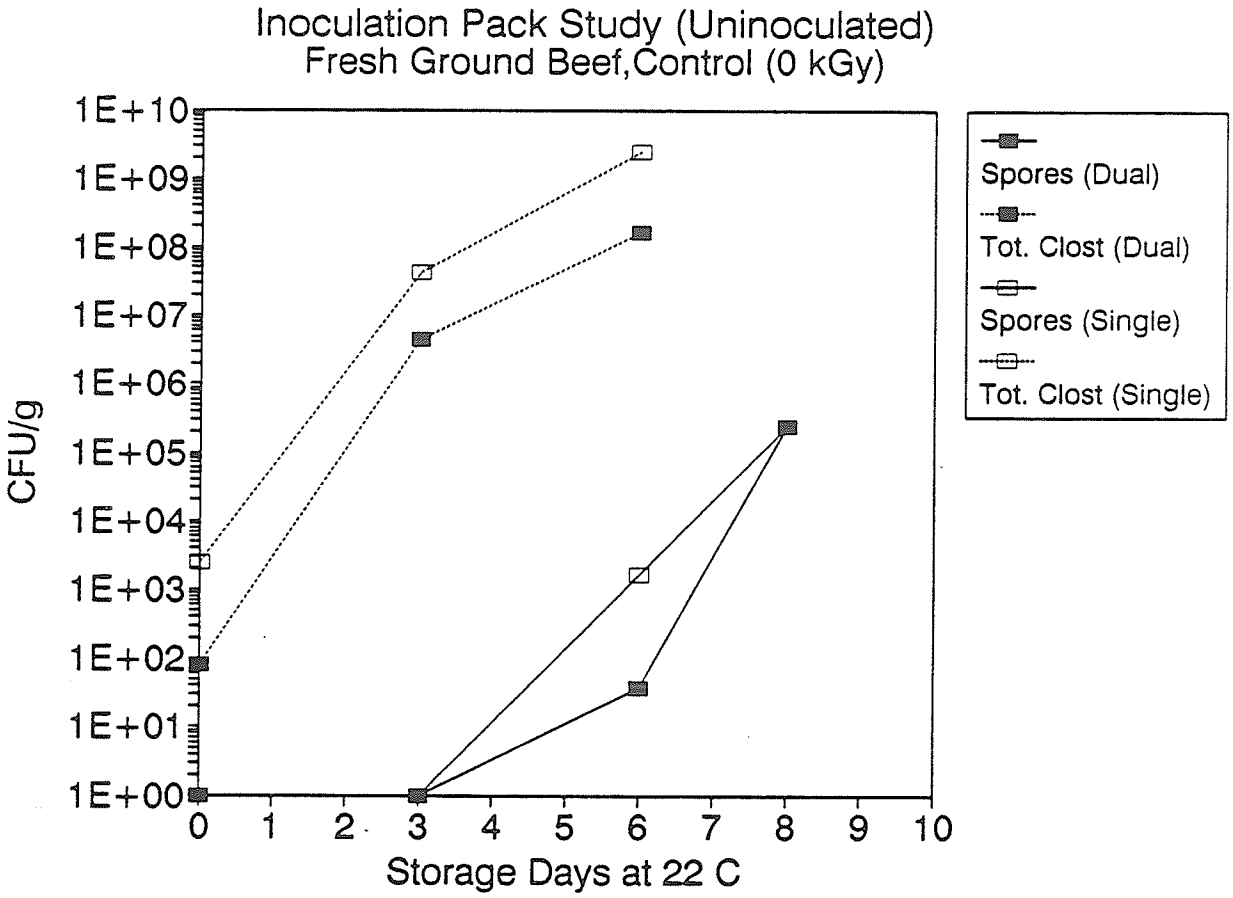

Inoculation Pack Study
Fresh Ground Beef,Control (0 kGy)
CFU/g
1E+10
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
0
1
2
3
4
5
6
7
8
9
10
Storage Days at 22 C
Spores (Dual)
Tot. Clost (Dual)
Spores (Single)
Tot. Clost (Single)

Figure 32.


Inoculation Pack Study (Uninoculated)
Fresh Ground Beef,Control (0 kGy)
CFU/g
1E+10
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
1E+00
0
1
2
3
4
5
6
7
8
9
10
Storage Days at 22 C
Spores (Dual)
Tot. Clost (Dual)
Spores (Single)
Tot. Clost (Single)

There was minimal interference in the clostridial spore assay. Figures 33 and 34 show that there were no colonies monitored on the clostridial spore assay for uninoculated samples until day 6 of storage in either packaging condition. There were greater than 4 $\log_{10}$ more colonies in inoculated versus uninoculated patties at this point. On day 8 there was at least a 1 $\log_{10}$ difference between the inoculated and uninoculated patties. The contribution of the inoculum to the clostridial spore assay was unmistakable, over the 8 storage days monitored.

The APC of inoculated patties packaged in either packaging condition on day 0 was approximately $4x10^6$/g (Fig. 35). The APC increased to greater than $1x10^9$/g by day 3. The APC remained constant at this level between day 3 and day 8, with the single bag packaged samples having slightly greater APC counts than corresponding dual bag packaged samples. The $5x10^7$/g spoilage criteria was reached between day 1 and 2 in both packaging conditions. Thus the APC for unirradiated control samples were approximately 1 Log_{10} greater than the APC found for irradiated samples in test #1 and #2.

The LAB in inoculated patties increased from less than $1x10^6$/g on day 0 to approximately $1x10^8$/g on day 3 in both packaging conditions (Fig. 35). The LAB remained constant at this level until day 8 of storage. This result was the same as the result for irradiated samples in test #1 (Fig. 26) and test #2 (Fig. 29).

Figure 33.

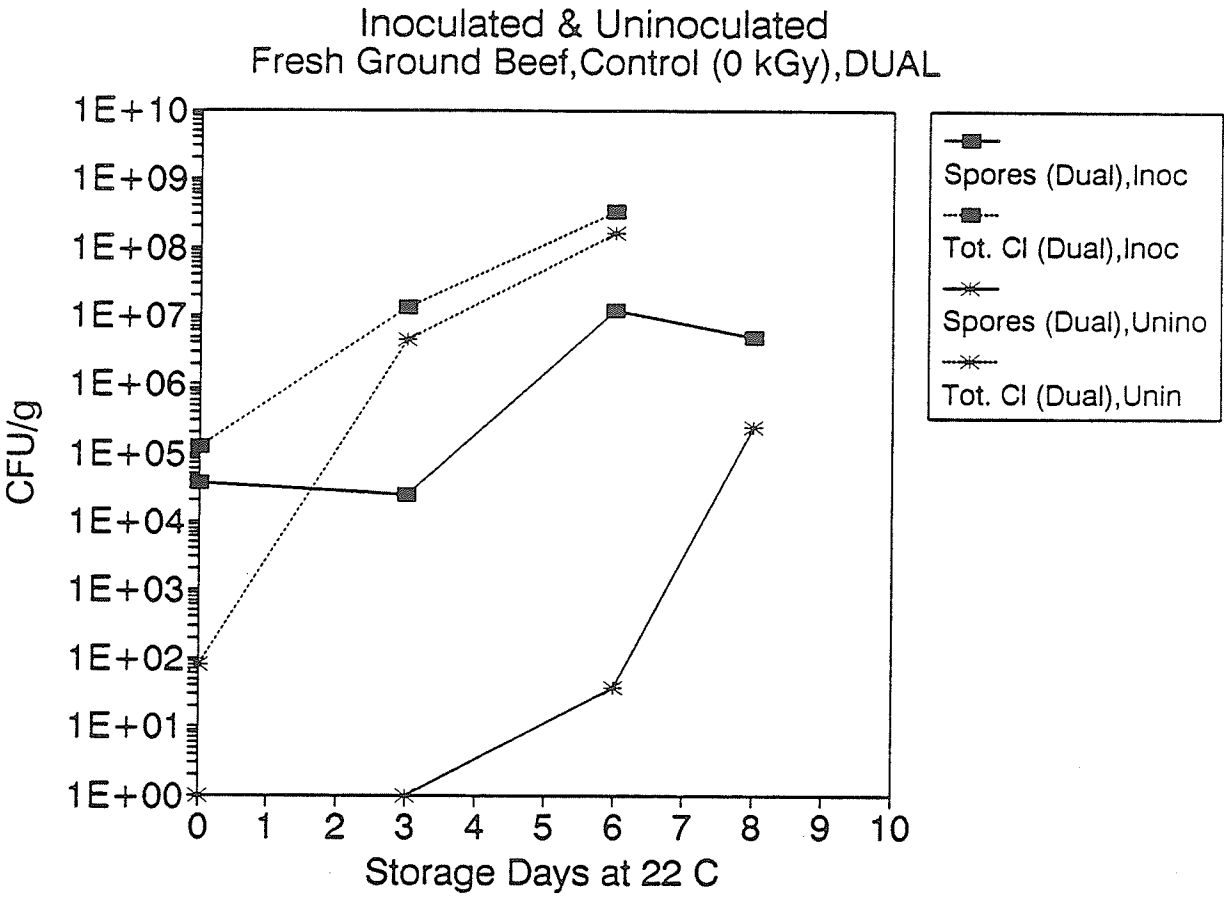

Inoculated & Uninoculated
Fresh Ground Beef,Control (0 kGy),DUAL
CFU/g
1E+10
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
1E+00
0 1 2 3 4 5 6 7 8 9 10
Storage Days at 22 C
Spores (Dual),Inoc
Tot. Cl (Dual),Inoc
Spores (Dual),Unino
Tot. Cl (Dual),Unin

Figure 34.

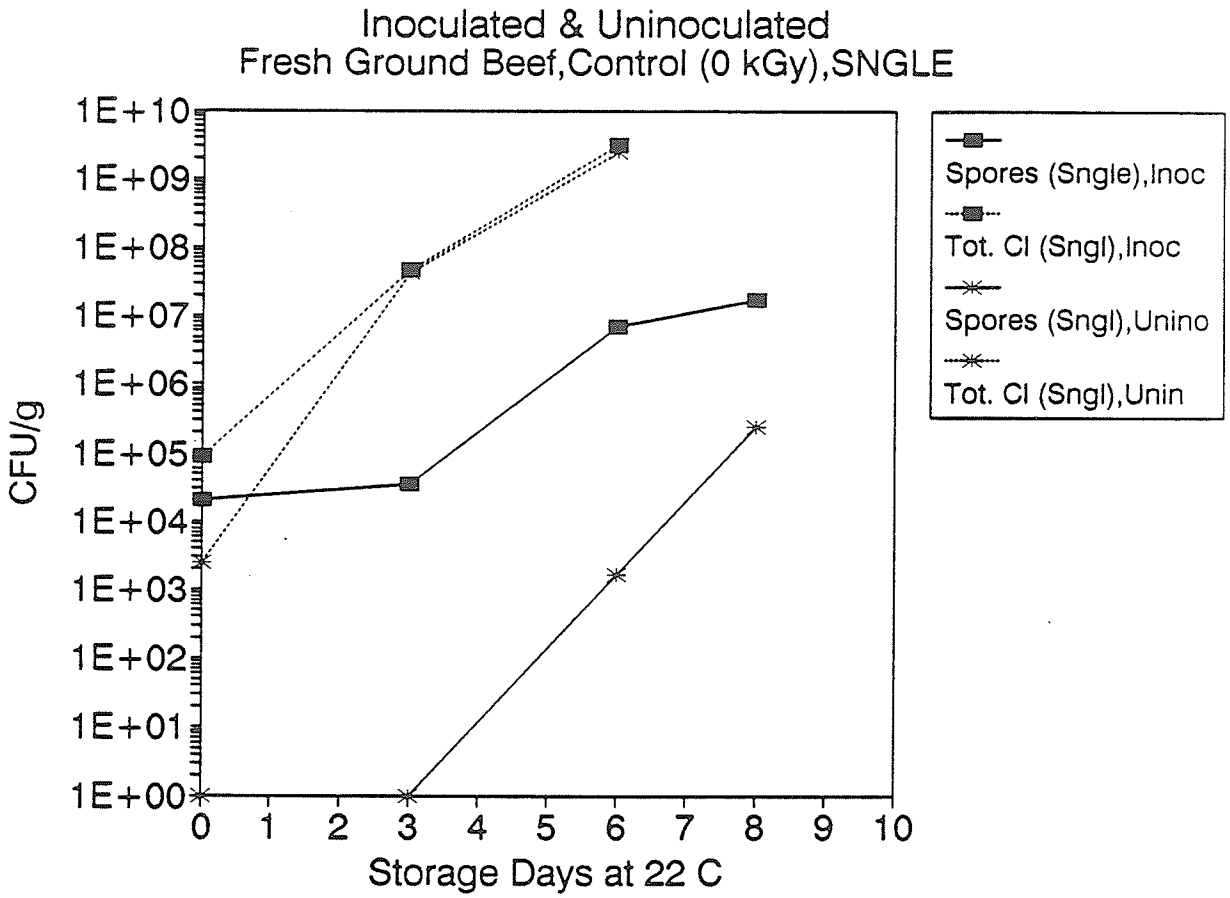
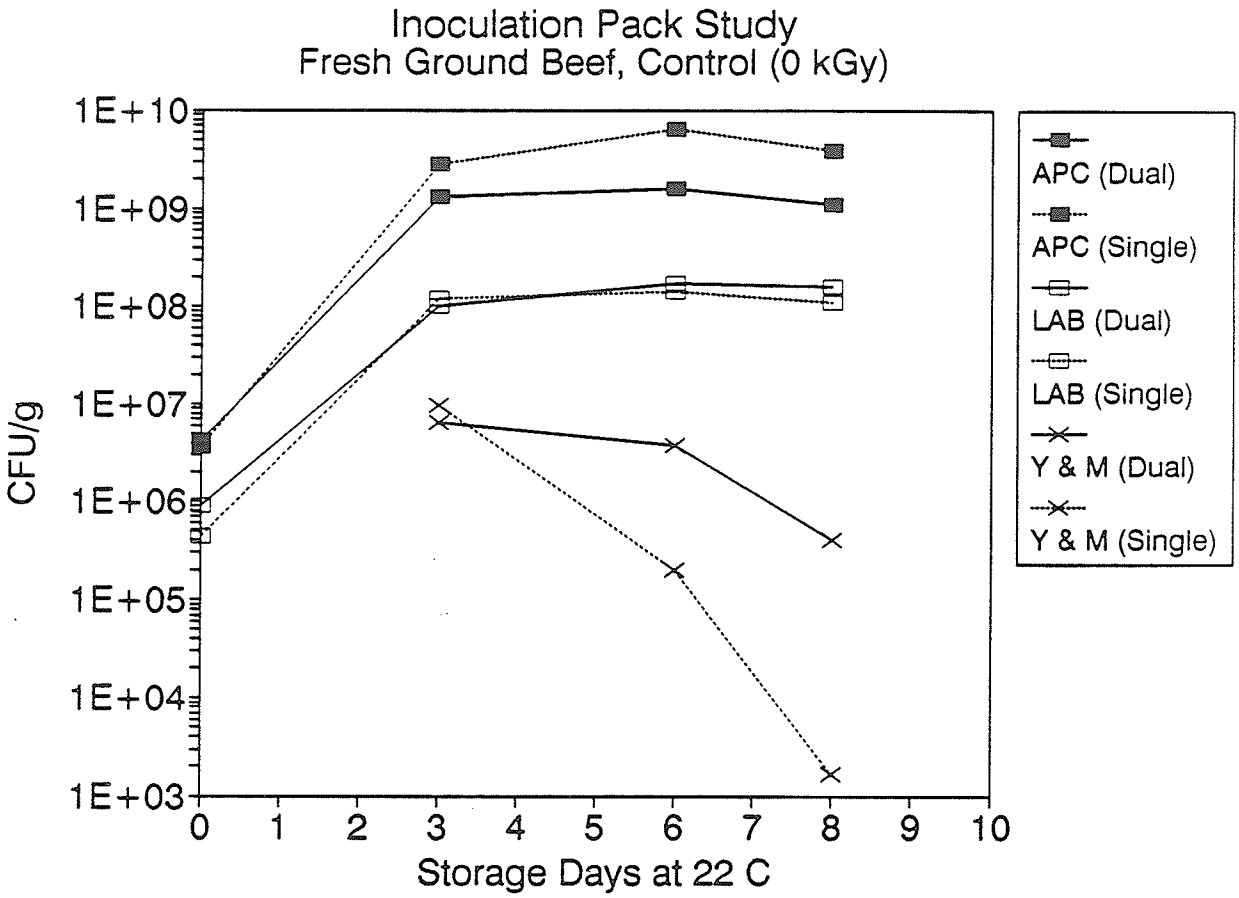

Inoculated & Uninoculated
Fresh Ground Beef,Control (0 kGy),SNGLE
CFU/g
1E+10
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
1E+00
0 1 2 3 4 5 6 7 8 9 10
Storage Days at 22 C
Spores (Sngle),Inoc
Tot. Cl (Sngl),Inoc
Spores (Sngl),Unino
Tot. Cl (Sngl),Unin

Figure 35.


Inoculation Pack Study
Fresh Ground Beef, Control (0 kGy)
CFU/g
1E+10
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
0
1
2
3
4
5
6
7
8
9
10
Storage Days at 22 C
APC (Dual)
APC (Single)
LAB (Dual)
LAB (Single)
Y & M (Dual)
Y & M (Single)

The Y&M in inoculated patties stored in either packaging condition reached just under $1x10^7$/g by day 3 (Fig. 35). (The initial Y&M content of uninoculated patties on day 0 was about $2x10^5$/g). By day 8, the Y&M count declined to less than $1x10^6$/g and an estimated $2x10^3$/g, for dual and single bag packaged samples, respectively. By comparison in irradiated patties, Y&M increased from $3x10^4$/g on day 0 to approximately $1x10^8$/g by day 3, stayed at this level until day 6, followed by a drop to under $1x10^6$/g by day 8 (Fig. 29).

(Inoculated - Uninoculated Comparison)

The APC, LAB, and Y&M growth curves for inoculated and uninoculated patties stored in dual and single bag packages are shown in Figs. 36-38. The growth curves for inoculated and uninoculated patties monitored for APC (Fig. 36) and LAB (Fig. 37) in similar packaging are almost identical. There were slight differences between the inoculated and uninoculated patties' Y&M growth curves (Fig. 38). The patty pH for inoculated and uninoculated patties in both dual and single bag packaging are shown in Fig. 39 and Fig. 40. The curves for inoculated dual and single bag packaged samples are similar to their corresponding uninoculated samples. This shows there was no contamination or change in microflora of the patties due to inoculation of spores. The pH for dual bag packaged samples by day 6 of storage was about 6.7, for inoculated and uninoculated samples (Fig. 39). The pH for single packaged samples by this time was approximately 8.0 (Fig. 40).

From the results for unirradiated control patties stored at 22°C in dual and single bag packaging, it could not be determined when there was growth in total clostridial due to interfering colonies on this assay.

The possible interfering black colony forming organism present in the natural background microflora of this batch of meat may be a Pseudomonas spp. Lin et al. (1977) found hydrogen sulfide producing pseudomonads in ground beef, as monitored on Peptone Iron Agar. The production of hydrogen sulfide by an organism would result in a black colony on our SFP based medium. A 2.5 kGy irradiation dose would eliminate pseudomonads from ground beef, thus explaining the lack of interfering black colonies on the 2.5 kGy irradiated samples. Proper isolation and identification of the interfering organism(s) should be conducted, if further modification to the medium is desired to allow this medium to be effective in unirradiated ground beef as well as radurized ground beef.

5.3.4.2 Storage at 15°C

The results of the inoculation pack study with irradiated and unirradiated control samples stored at 15°C in both packaging conditions are given in Table 21. The irradiated samples were monitored after 25 and 29 days of storage. The unirradiated control samples were monitored after 26 days of storage.

Figure 36.

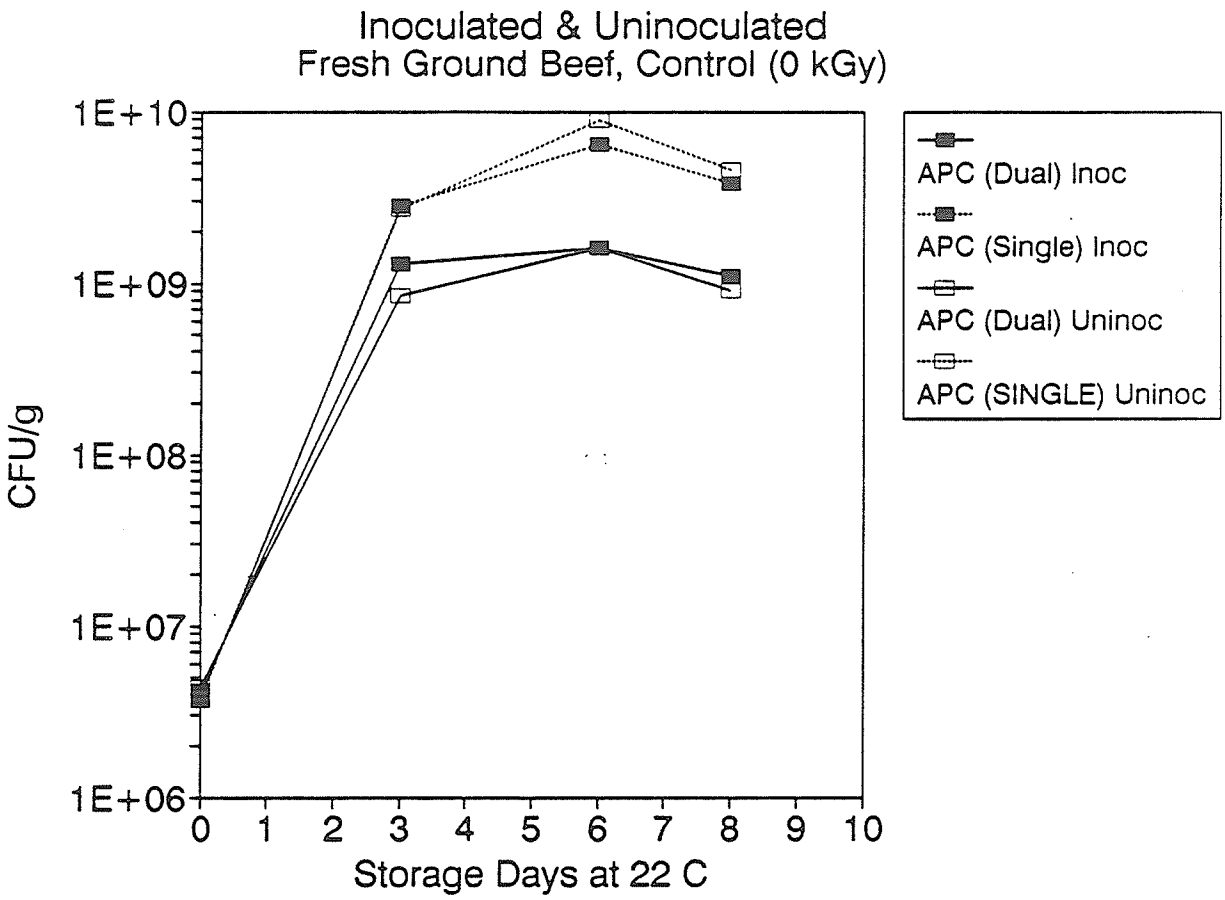

Inoculated & Uninoculated
Fresh Ground Beef, Control (0 kGy)
1E+10
1E+09
1E+08
1E+07
1E+06
CFU/g
0 1 2 3 4 5 6 7 8 9 10
Storage Days at 22 C
APC (Dual) Inoc
APC (Single) Inoc
APC (Dual) Uninoc
APC (SINGLE) Uninoc

Figure 37.

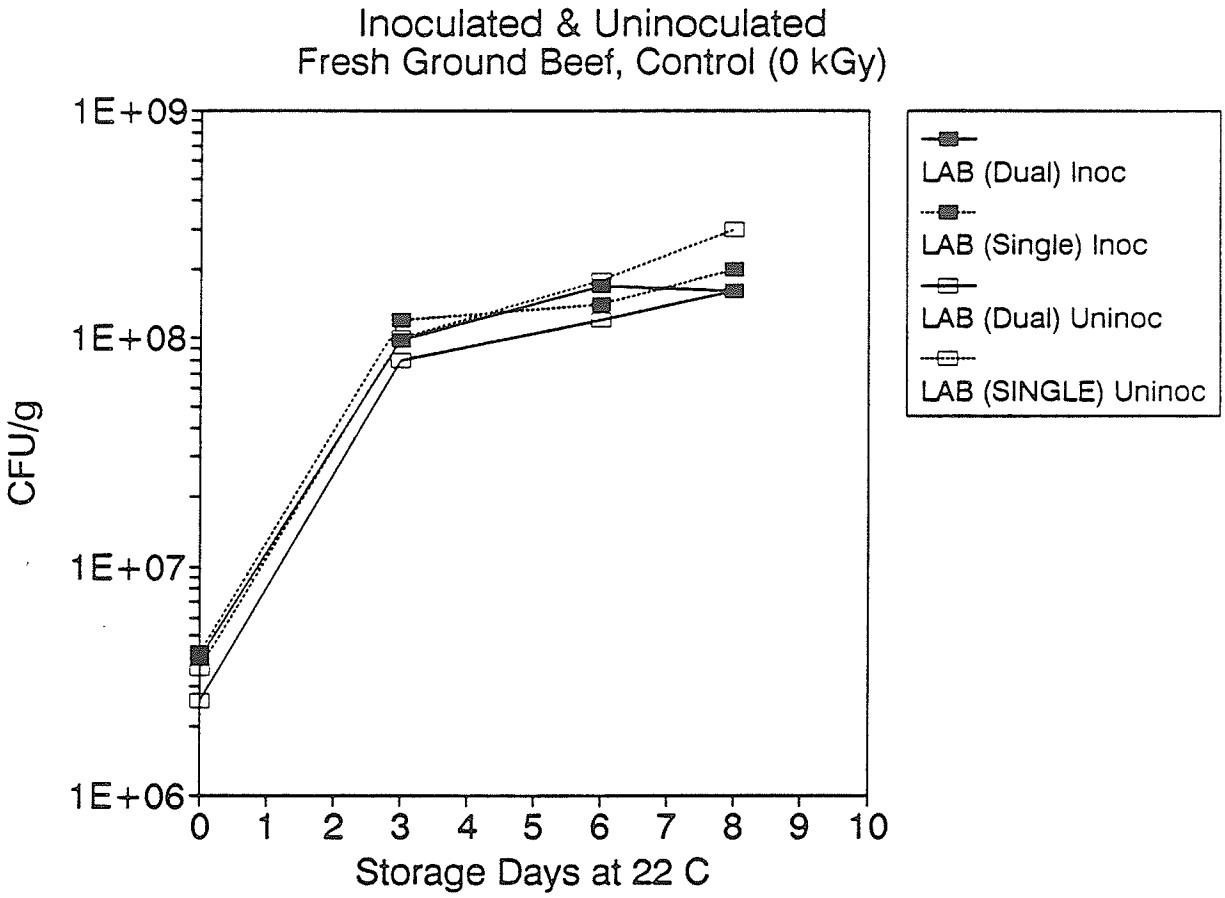

Inoculated & Uninoculated
Fresh Ground Beef, Control (0 kGy)
1E+09
1E+08
1E+07
1E+06
CFU/g
0 1 2 3 4 5 6 7 8 9 10
Storage Days at 22 C
LAB (Dual) Inoc
LAB (Single) Inoc
LAB (Dual) Uninoc
LAB (SINGLE) Uninoc

Figure 38.

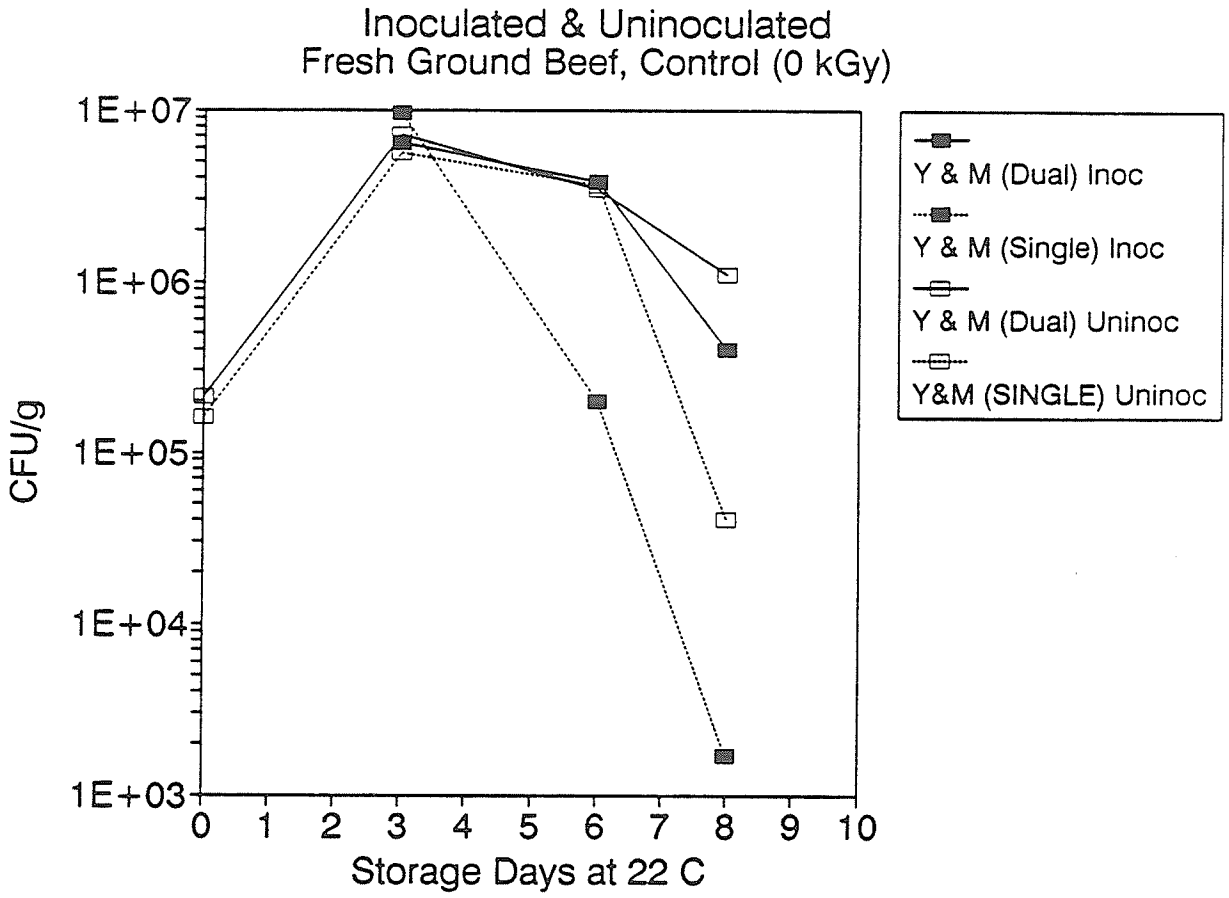

Inoculated & Uninoculated
Fresh Ground Beef, Control (0 kGy)
CFU/g
1E+07
1E+06
1E+05
1E+04
1E+03
0 1 2 3 4 5 6 7 8 9 10
Storage Days at 22 C
Y & M (Dual) Inoc
Y & M (Single) Inoc
Y & M (Dual) Uninoc
Y&M (SINGLE) Uninoc

Figure 39.

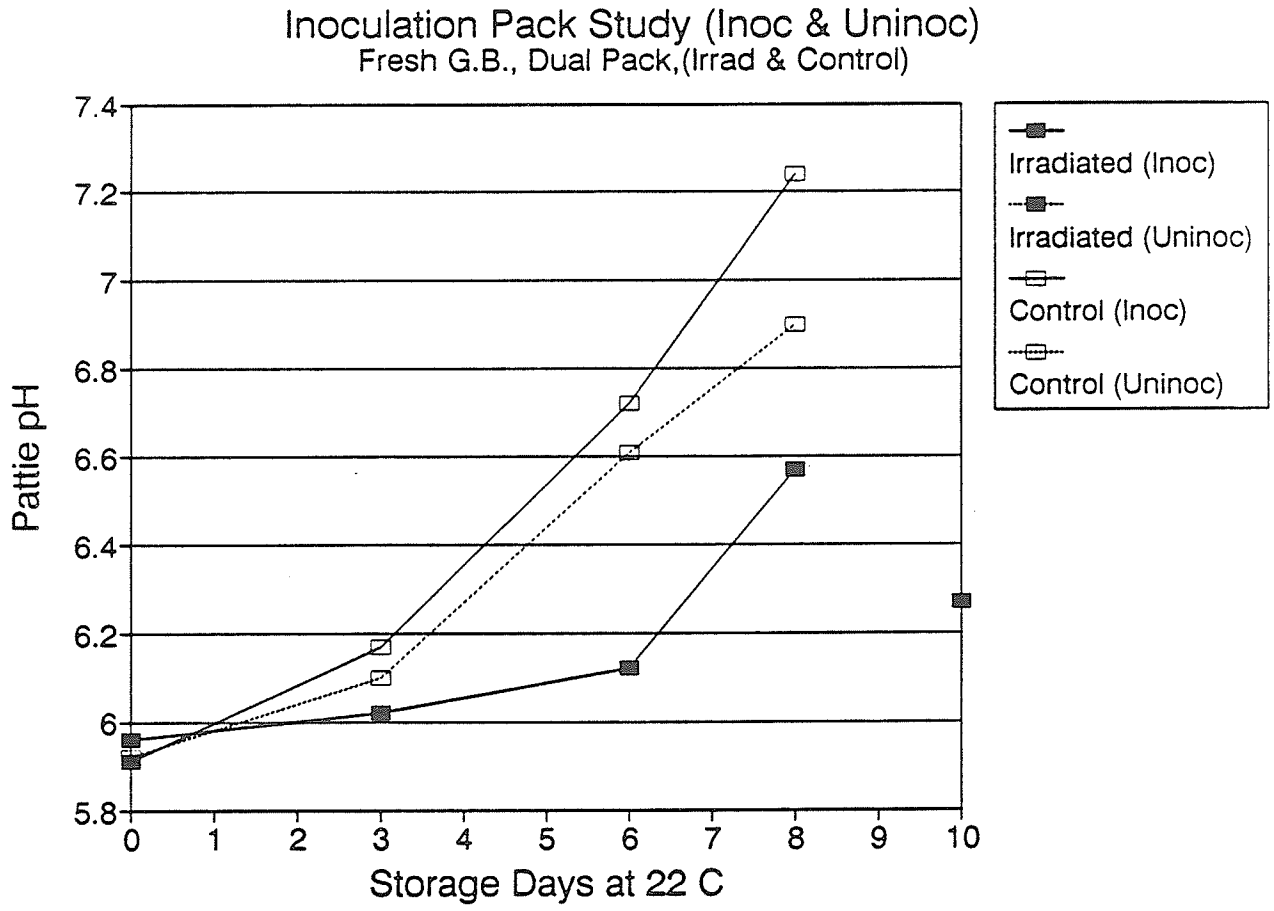
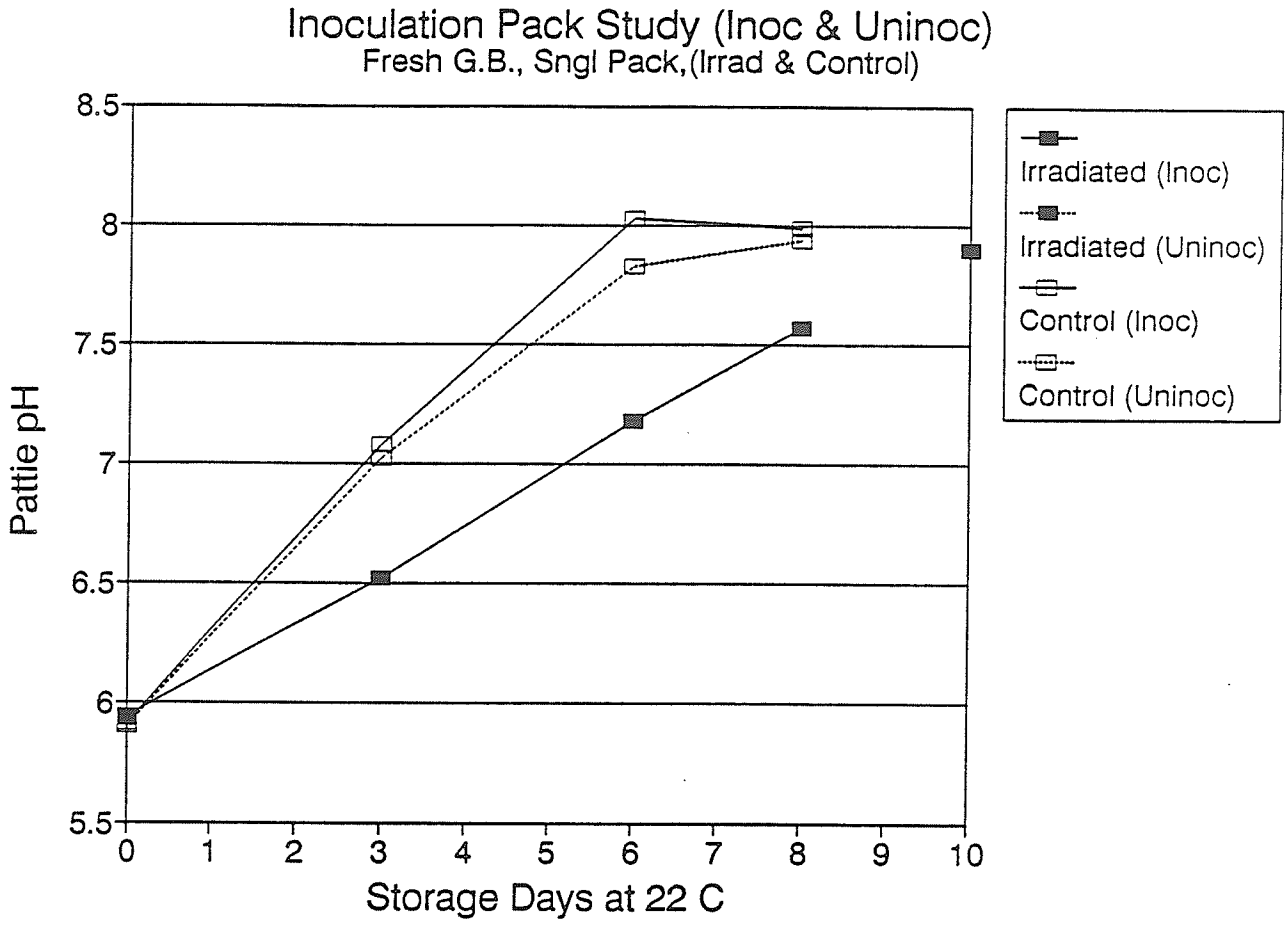

Inoculation Pack Study (Inoc & Uninoc)
Fresh G.B., Dual Pack,(Irrad & Control)
Pattie pH
7.4
7.2
7
6.8
6.6
6.4
6.2
6
5.8
0
1
2
3
4
5
6
7
8
9
10
Storage Days at 22 C
Irradiated (Inoc)
Irradiated (Uninoc)
Control (Inoc)
Control (Uninoc)

Figure 40.


Inoculation Pack Study (Inoc & Uninoc)
Fresh G.B., Sngl Pack,(Irrad & Control)
Pattie pH
8.5
8
7.5
7
6.5
6
5.5
0
1
2
3
4
5
6
7
8
9
10
Storage Days at 22 C
Irradiated (Inoc)
Irradiated (Uninoc)
Control (Inoc)
Control (Uninoc)

(Irradiated 2.5 kGy)

The total clostridia in inoculated dual and single bag packages did not increase from the initial level of approximately $1x10^4$/g after 25 and 29 days of storage. On day 29 there were $2.7x10^4$/g and $1.2x10^4$/g in dual and single bag packaging, respectively (Table 21).

The clostridial spores present in inoculated dual and single bag packaged samples decreased slightly from the initial level of approximately $1x10^4$/g after 25 and 29 days of storage. The dual packaged samples had $6.9x10^3$/g after day 25 and $7.5x10^3$/g after day 29 (Table 21). The single bag packaged samples were slightly lower with $2.2x10^3$/g after day 25 and $1.6x10^3$/g after day 29.

The APC of inoculated and uninoculated patties in both packaging conditions were greater than $1x10^8$/g on day 25 and 29. Thus the detectable spoilage level of $5x10^7$/g APC has been surpassed by these dates, with no clostridial growth in either packaging condition.

(Unirradiated Control)

The total clostridia assay in inoculated dual and single bag packages contained an estimated $5x10^8$/g and $7x10^8$/g, of tiny black (non-typical sporogenes) colonies, respectively on day 26 (Table 21). The corresponding uninoculated samples contained similar concentrations of this interfering organism, thus the contribution of the inoculum can not be determined.

The number of black colonies monitored with the clostridial spore assay in inoculated dual and single bag packaged samples on day 26 were

The number of black colonies monitored with the clostridial spore assay in inoculated dual and single bag packaged samples on day 26 were approximately $6x10^5$/g and $8x10^5$/g, respectively. There were approximately $2x10^5$/g of these colonies in uninoculated patties stored in either packaging condition. The high number of colonies in the uninoculated samples make it impossible to determine the contribution of the inoculum. There were also white (non-typical C. sporogenes) colonies detected in inoculated and uninoculated samples with this assay. White colonies had never been observed on the clostridial spore assay for any irradiated samples or unirradiated control samples on day 0.

The APC on day 26 for inoculated and corresponding uninoculated samples stored in dual and single packaging were approximately the same. The APC for dual and single bag packaged samples on day 26 was approximately $7x10^8$/g and $2x10^9$/g, respectively. Thus the $5x10^7$/g APC spoilage criteria was surpassed prior to this date.

The pH for dual and single bag packaged samples on day 26 was approximately 7.1 and 8.1, respectively.

5.3.4.3 Storage at 4°C

None of the samples stored at 4°C were inoculated. Patties stored in dual and single bag packaging were evaluated on days 0, 7, 15, and 21 of storage. The data for day 0 samples were taken from the common day 0 uninoculated values (Table 19).

Table 21. Inoculation Pack Study, Fresh Ground Beef Test #2, Initial and Storage at 15°C

Day	Inoc	Dose (kGy)	Pack	pH	Clostridium Sporogenes Total/mL	Clostridium Sporogenes Spores/mL	APC (/g)
0	No	0	Dual	5.92	$8x10^{1}$E*	0	$4.4x10^{6}$
0	No	0	Single	5.90	$2.4x10^{3}$*	0	$3.8x10^{6}$
0	No	2.5	Dual	5.91	0	0	$4.8x10^{3}$
0	No	2.5	Single	5.93	0	0	$2.5x10^{3}$
0	Yes	0	Dual	5.91	$1.2x10^{5}$	$3.7x10^{4}$	$4.2x10^{6}$
0	Yes	0	Single	5.91	$9.0x10^{4}$	$2.0x10^{4}$	$3.7x10^{6}$
0	Yes	2.5	Dual	5.96	$1.2x10^{4}$	$1.8x10^{4}$	$2.2x10^{3}$
0	Yes	2.5	Single	5.94	$1.2x10^{4}$	$1.2x10^{4}$	$1.9x10^{3}$
25	No	2.5	Dual	6.28	0	0	$1.6x10^{8}$
25	No	2.5	Single	8.22	0	0	$3.4x10^{8}$
25	Yes	2.5	Dual	6.20	$2.4x10^{4}$	$6.9x10^{3}$	$2.0x10^{8}$
25	Yes	2.5	Single	8.34	$1.2x10^{4}$	$2.2x10^{3}$	$2.6x10^{8}$
26	No	0	Dual	7.10	$5.0x10^{8}$E*	$2.2x10^{5}$!	$7.4x10^{8}$
26	No	0	Single	8.01	$8.0x10^{8}$E*	$2.1x10^{5}$!	$2.7x10^{9}$
26	Yes	0	Dual	7.05	$5.0x10^{8}$E*	$6.4x10^{5}$!	$7.2x10^{8}$
26	Yes	0	Single	8.28	$7.2x10^{8}$E*	$8.2x10^{5}$!	$1.9x10^{9}$
29	No	2.5	Dual	6.26	0 $*	0	$3.8x10^{8}$
29	No	2.5	Single	8.14	0	0	$4.4x10^{8}$
29	Yes	2.5	Dual	6.04	$2.7x10^{4}$	$7.5x10^{3}$	$1.5x10^{8}$
29	Yes	2.5	Single	8.34	$1.2x10^{4}$	$1.6x10^{3}$	$2.5x10^{8}$

E Estimated Count
* Tiny black (non-typical C. sporogenes) colonies
! Estimated Count, not including White colonies also visible
$ No typical C. sporogenes colonies observed

The APC of irradiated (2.5 kGy) and unirradiated control patties stored at 4°C are shown in Fig. 41. The APC of irradiated dual and single bag packaged patties was approximately $4x10^4$/g on day 0 (Fig. 41 and Table 19). This is about a 3 Log10 cycle reduction from the unirradiated control's APC of approximately $4x10^6$/g. In both packaging conditions the APC of irradiated samples declined to approximately $5x10^2$/g by day 7. Between day 7 and day 15 there were approximately 4 and 5 Log10 increases to $3x10^6$/g and $6x10^7$/g for dual and single bag packaged samples, respectively. Patties in both packaging conditions had a declining APC between day 15 and day 21 of storage. The dual and single bag packaged samples declined to $2x10^5$/g and $4x10^6$/g, respectively. The dual packaged patties never reached the $5x10^7$/g APC spoilage level. The single bag packaged sample reached this spoilage level on approximately day 15 of storage.

The APC of unirradiated control patties increased from the day 0 level of $4x10^6$/g by day 7 of storage (Fig. 41 and Table 19). The day 7 APC of dual bag packaged patties was $1.2x10^7$/g. The single bag packaged patties had reached $1.2x10^8$/g by this time. Single bag packaged patties maintained an APC of greater than $1x10^8$/g up to day 21 of storage. The APC of dual bag packaged patties increased to approximately $1x10^8$/g by day 15, followed by a decline to $4x10^7$/g on day 21. The APC spoilage criteria was reached on approximately day 12 and day 3 for dual and single bag packaged unirradiated control patties, respectively.

The LAB of irradiated and unirradiated control, dual and single bag packaged patties are shown in Fig. 42. The LAB of irradiated dual and single bag packaged patties is approximately $7x10^2$/g on day 0 (Fig. 42). This is about a 3 Log10 cycle reduction from the unirradiated control's LAB of approximately $7x10^5$/g. In both packaging conditions the LAB of irradiated samples declined by day 7, with single bag packaged samples showing the greater decline. Between day 7 and 21 the LAB of storage patties in both packaging condition increased, with approximately $2x10^5$/g on day 21.

The LAB of unirradiated control patties in both packaging conditions increased from the day 0 level of $7x10^5$/g to approximately $8x10^6$/g on day 7 (Fig. 42). Between days 7 and 21 there were very slight increases in LAB for patties in both packaging conditions.

The Y&M of irradiated and unirradiated controls, dual and single bag packaged patties are shown in Fig.42. The day 0 Y&M content of irradiated patties is approximately $3x10^4$/g (Fig. 42 and Table 19). This is approximately a 1 Log10 cycle reduction from the uninoculated control patties' Y&M of $2x10^5$/g. There is a rapid increase in Y&M of patties stored in both packaging conditions between day 0 and day 21. By day 15 of storage the irradiated patties stored in dual and single bag packaging equalled or exceeded the Y&M content of the corresponding uninoculated control patties. Levels in single bag packaged samples were always greater than in corresponding dual bag packaged samples.

Figure 41.

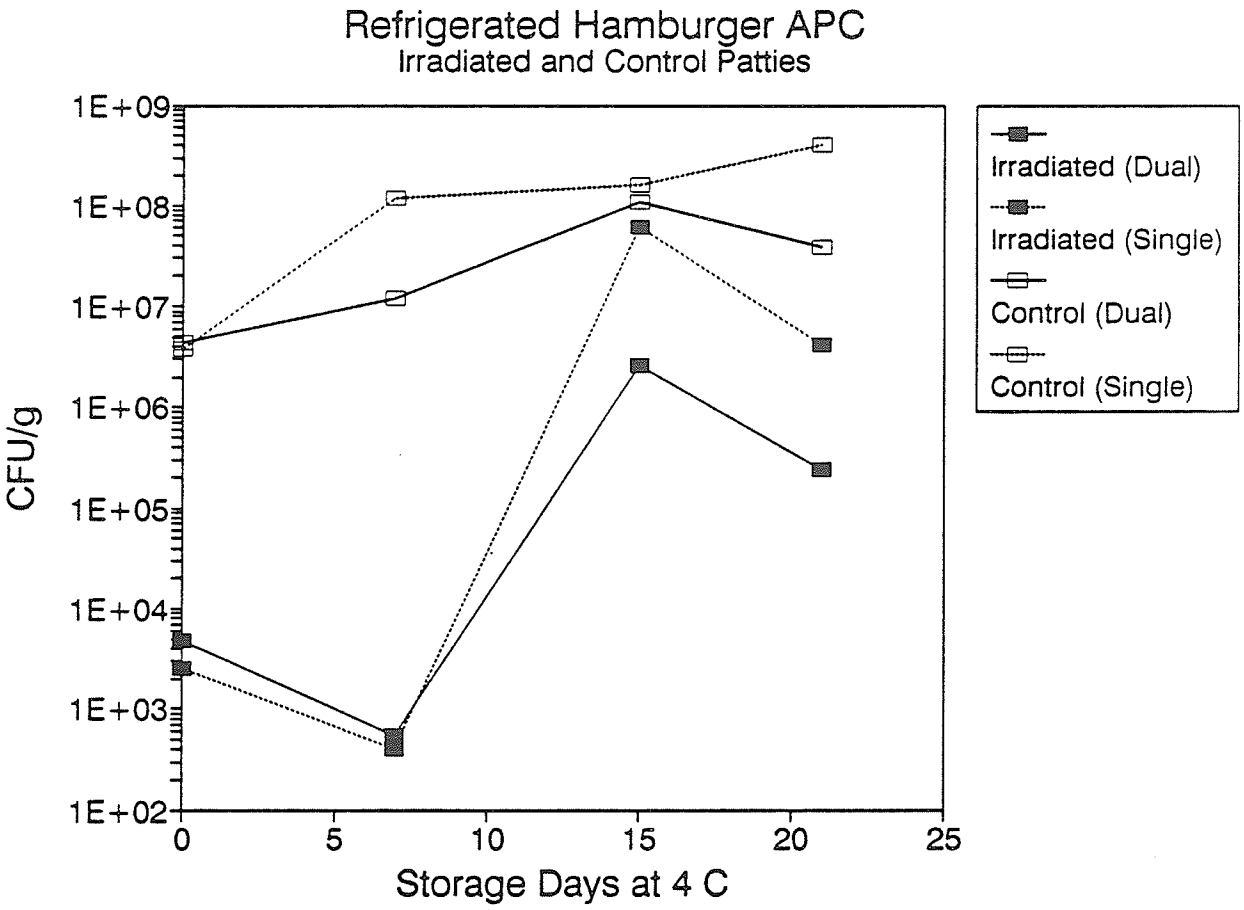
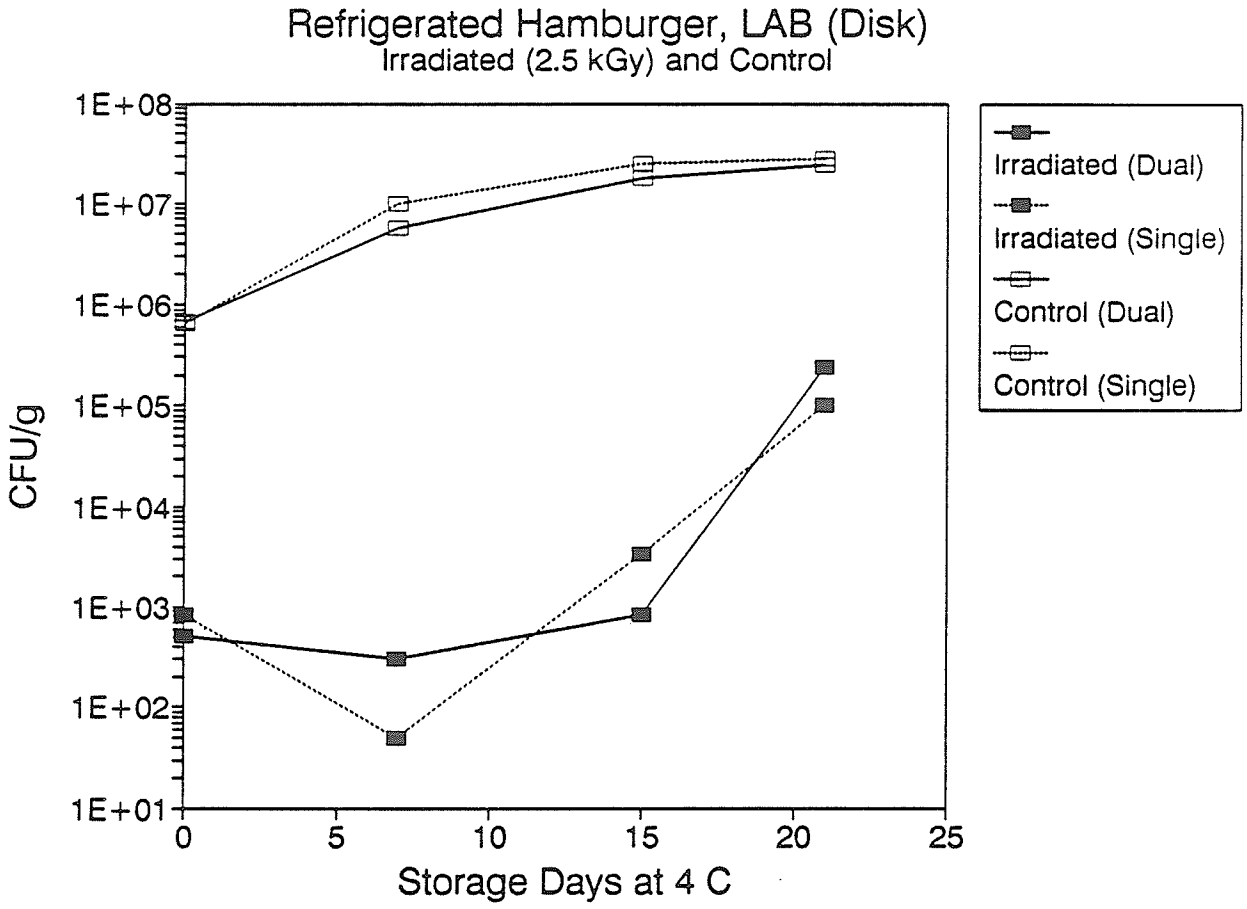

Refrigerated Hamburger APC
Irradiated and Control Patties
CFU/g
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
0
5
10
15
20
25
Storage Days at 4 C
Irradiated (Dual)
Irradiated (Single)
Control (Dual)
Control (Single)

Figure 42.


Refrigerated Hamburger, LAB (Disk)
Irradiated (2.5 kGy) and Control
CFU/g
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
0
5
10
15
20
25
Storage Days at 4 C
Irradiated (Dual)
Irradiated (Single)
Control (Dual)
Control (Single)

The Y&M of unirradiated control patties stored in both packaging conditions were similar. The day 0 level of $2x10^5$/g increases rapidly to approximately $1x10^7$/g by day 7 (Fig. 43). The Y&M content was maintained at this level until day 21. Thus Y&M content of irradiated patties is greater than unirradiated control patties after more than 15 days storage at 4°C. Fig. 44 shows that in irradiated patties compared to APC, or LAB-disk, Y&M is the dominant microflora of patties stored in either packaging condition. This is likely due to Y&M being more radiation resistant than organisms monitored in the other assays. Irradiation of patties reduced Y&M by less than 1 Log10 where as APC,and LAB-disk counts were reduced by approximately 3 Log10 cycles (Table 19). The lack of competition from other organisms in addition to the higher percentage of yeasts and moulds present in irradiated patties, leads to their dominance during storage at 4°C.

Figure 45 shows the psychrotrophs (PSY) and Y&M present in irradiated patties stored in dual and single bag packaging. There are more PSY and Y&M present in single bag packaged patties than dual bag packaged patties. The PSY growth curves for dual and single bag packaged patties superimposes on the corresponding Y&M curves. This indicates that the organisms being monitored with the selective Y&M assay are the same as those being monitored on the more general PSY assay. Thus the psychrotrophic organisms monitored are in fact yeast and mould. This was confirmed by the shape and size of colonies on the PSY assay, which were typical of yeast and mould.

Figure 43.

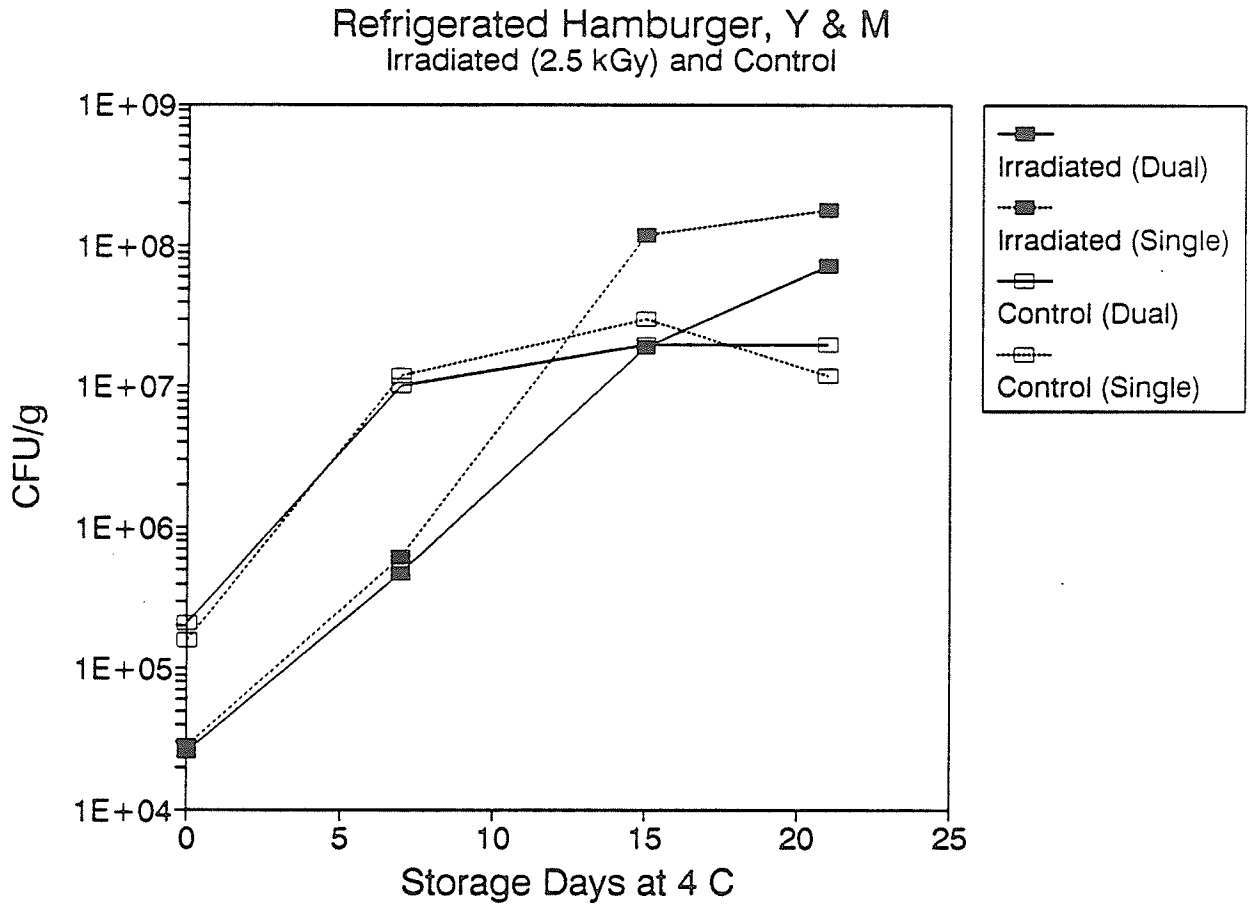

Refrigerated Hamburger, Y & M
Irradiated (2.5 kGy) and Control
CFU/g
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
0
5
10
15
20
25
Storage Days at 4 C
Irradiated (Dual)
Irradiated (Single)
Control (Dual)
Control (Single)

Figure 44.

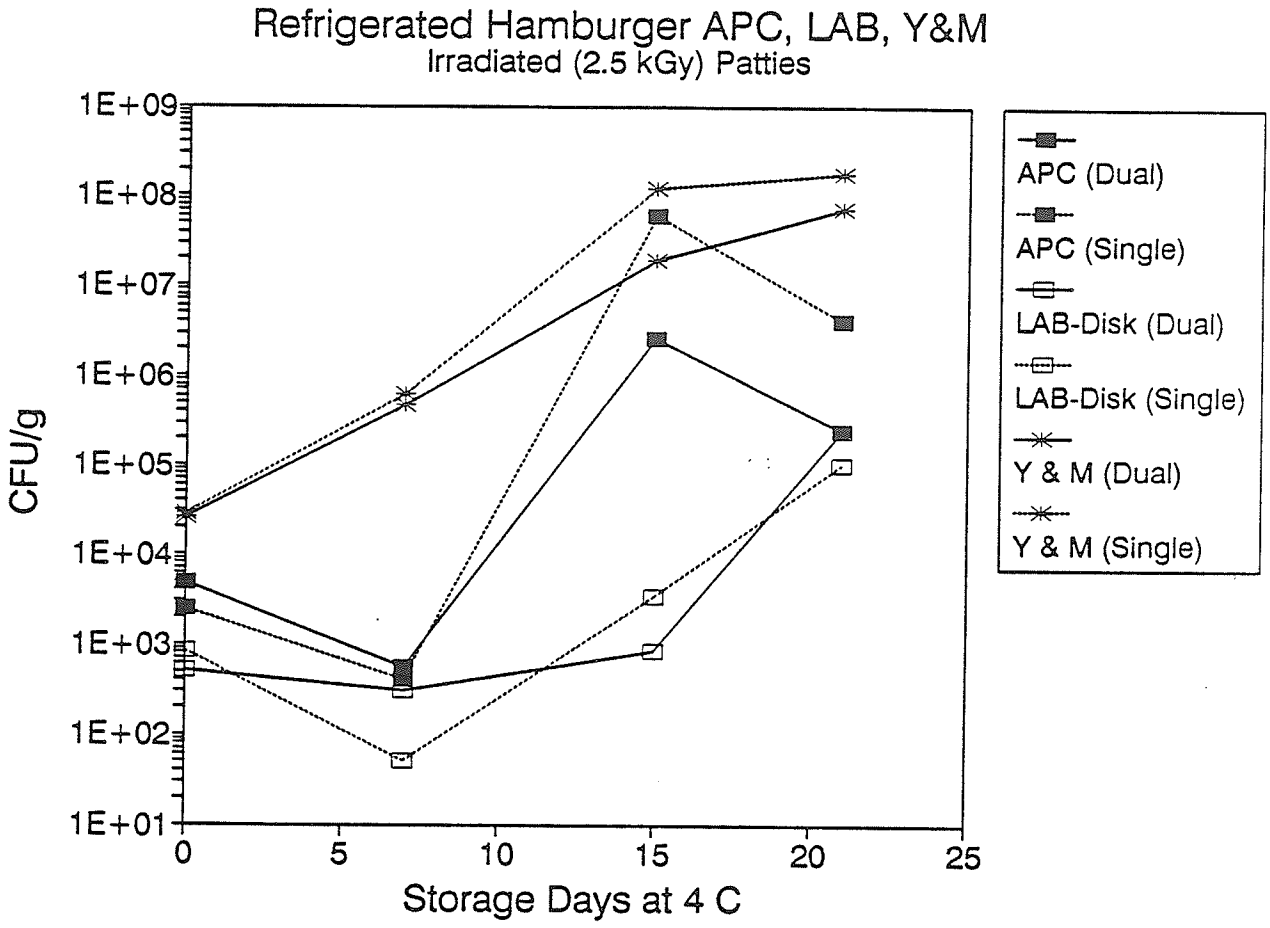
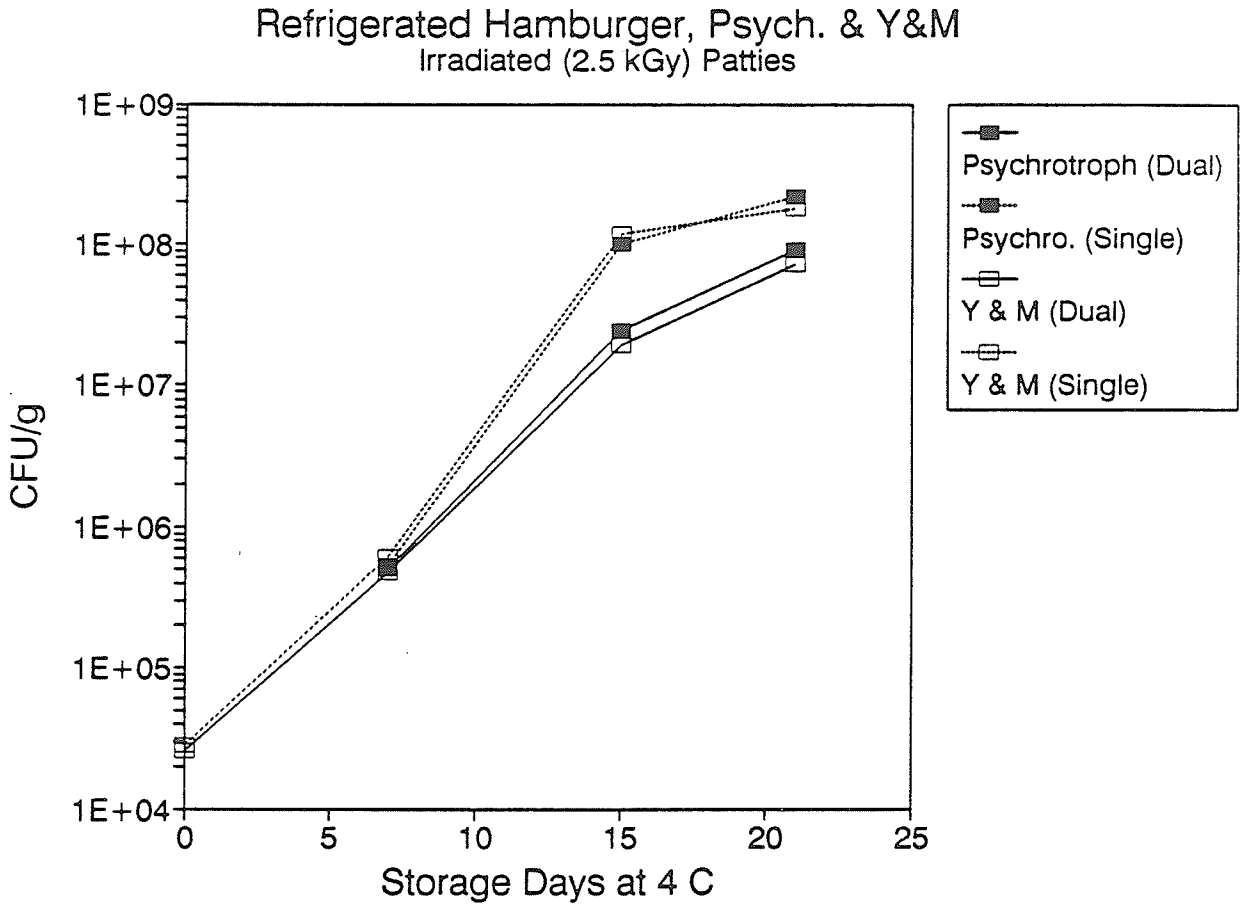

Refrigerated Hamburger APC, LAB, Y&M
Irradiated (2.5 kGy) Patties
CFU/g
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
0
5
10
15
20
25
Storage Days at 4 C
APC (Dual)
APC (Single)
LAB-Disk (Dual)
LAB-Disk (Single)
Y & M (Dual)
Y & M (Single)

Figure 45.

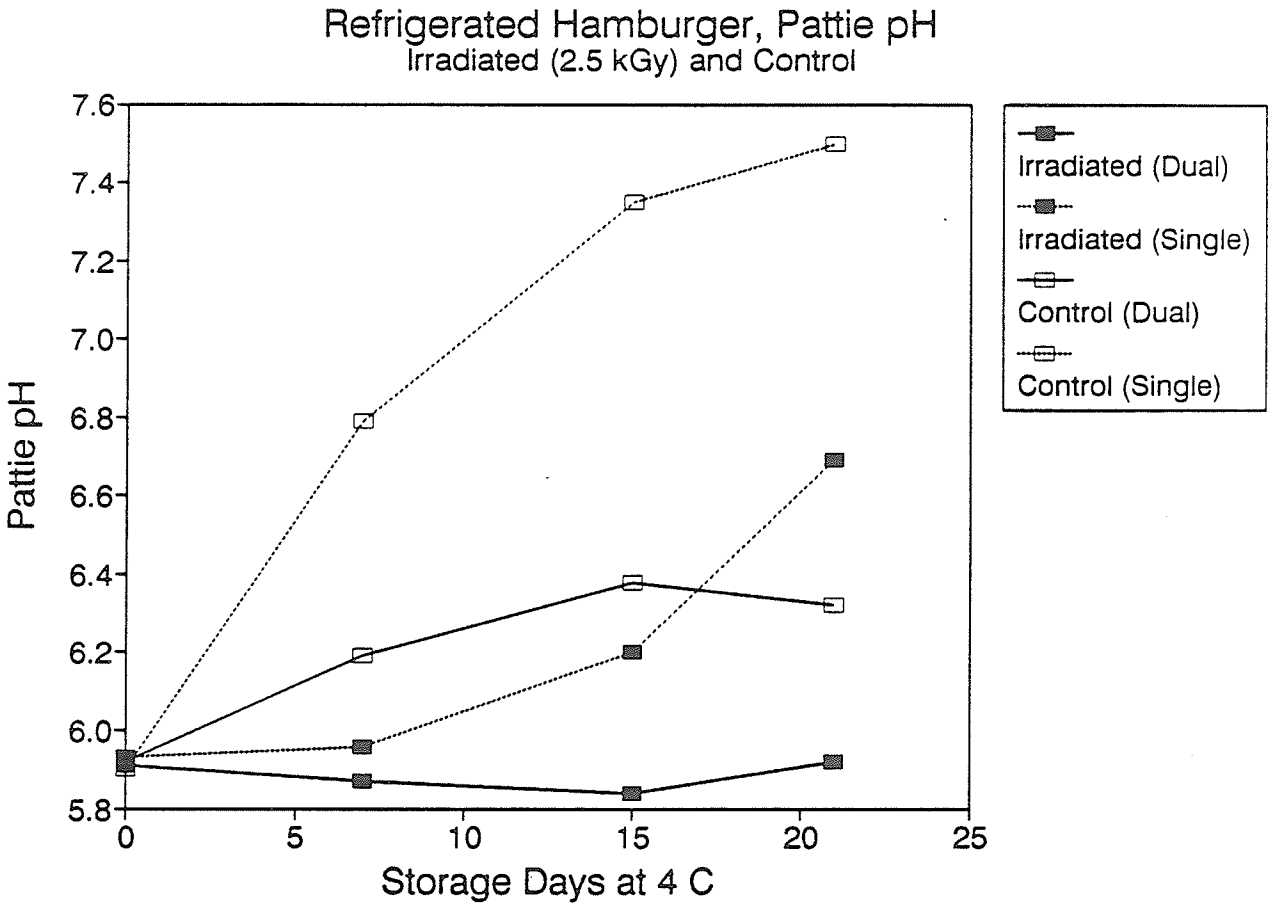

Refrigerated Hamburger, Psych. & Y&M
Irradiated (2.5 kGy) Patties
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
CFU/g
0
5
10
15
20
25
Storage Days at 4 C
Psychrotroph (Dual)
Psychro. (Single)
Y & M (Dual)
Y & M (Single)

The pH of irradiated and unirradiated inoculated patties stored in both types of packaging conditions at 4°C are shown in Figure 46. The patty pH on day 0 for irradiated and unirradiated control patties was approximately 5.9 (Fig. 46 and Table 19). The pH of the single bag packaged patties increased more rapidly than the corresponding dual bag packaged patties. By day 15 of storage the patty pH of dual bag packaged irradiated and unirradiated samples increased to approximately 6.2 and 5.8, respectively, while corresponding single bag packaged samples increased to 7.4 and 6.4. During this period of time the pH of unirradiated control samples increased more rapidly than corresponding irradiated samples similarly packaged. This demonstrates the reduction in organisms due to irradiation also yields a delay in pH increase. Between day 15 and 21 the pH of irradiated and unirradiated control patties stored in dual bag packaging, increased and decreased slightly, respectively. While all single bag packaged samples had substantial pH increases, there was no initial decrease in patty pH as seen at 22°C storage in test #1 (Fig. 46).

5.4 Shelf Life Extension

The refrigerated shelf life extension studies involved fresh ground beef, stored in dual or single bag packaging at 4°C unless otherwise stated. There was no inoculation of C. sporogenes spores. Samples were irradiated with the I-10/1 electron beam accelerator unless otherwise stated.

Figure 46.


Refrigerated Hamburger, Pattie pH
Irradiated (2.5 kGy) and Control
Pattie pH
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
0
5
10
15
20
25
Storage Days at 4 C
Irradiated (Dual)
Irradiated (Single)
Control (Dual)
Control (Single)

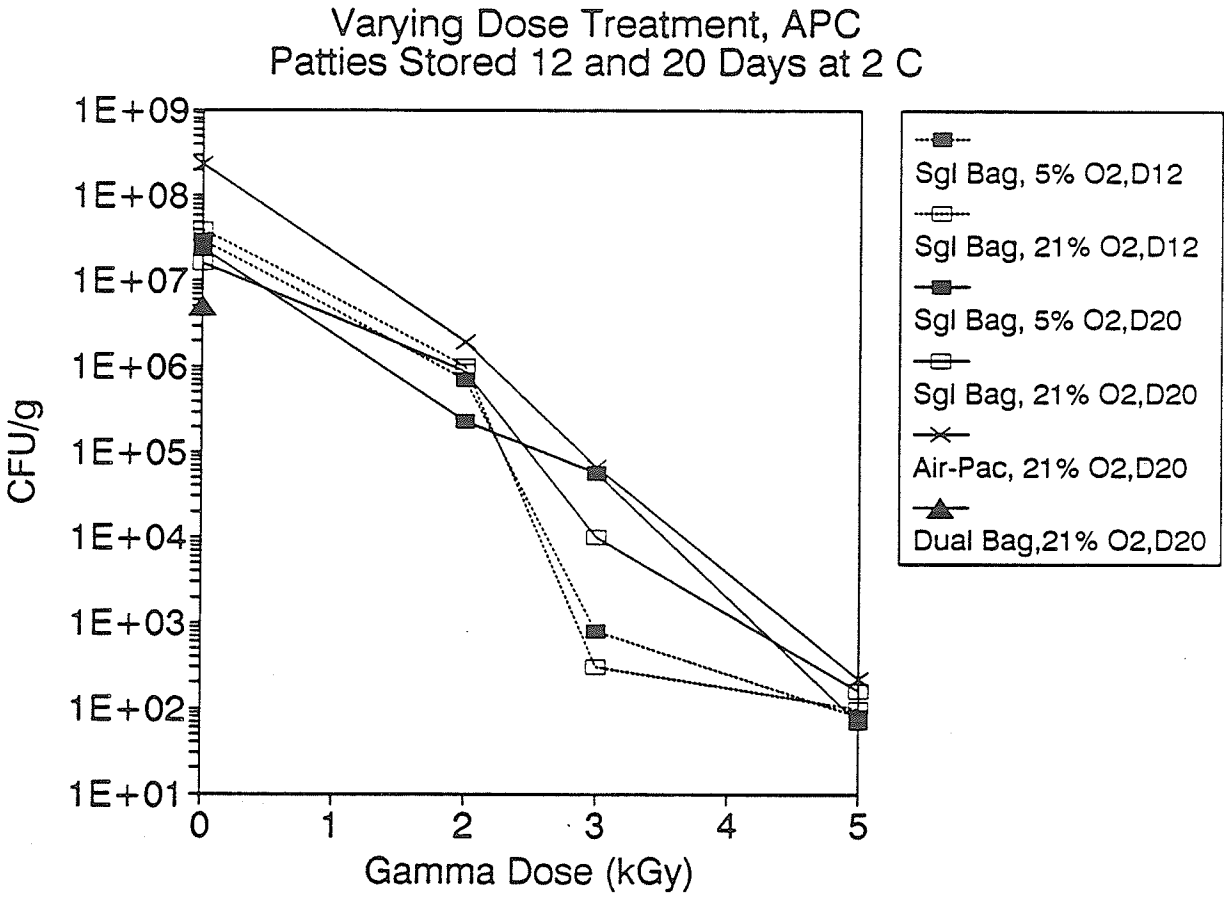
5.4.1 Dose Dependency Experiment

A dose dependency experiment was conducted to determine the effect of varying doses of irradiation (gamma) on patties stored for 12 and 20 days at 2°C. Patties were irradiated in dual bag packaging, or in air-packs where the inner oxygen permeable bags were not nitrogen backflushed. The outer barrier bag was removed from some of the patties and these patties were stored in the oxygen permeable inner bag at 2°C in a controlled atmosphere chamber (atmosbag) maintained at 5% oxygen or at ambient oxygen (21%). The aerobic plate count (APC), anaerobic plate count (AnPC), and psychrotrophic count (PSY) were evaluated on days 12 and 20 of storage under the conditions listed.

The results for the APC on days 12 and 20 of storage are shown in Fig 47. Increased dose resulted in decreased APC on days 12 and 20. The APC was not affected by the oxygen concentration surrounding the stored package. The air-packaged patties had higher APC's than the nitrogen backflushed samples. At the 0, 2, and 5 kGy dose levels there was not a substantial increase in APC between day 12 and day 20 with the single bag packaged patties. At the 3 kGy level there was a 2 Log10 increase in APC when storage was extended to 20 days.

An irradiation dose level of 2.5 kGy was utilized in all subsequent shelf life extension experiments. This dose appears to offer adequate extension of shelf life compared to unirradiated control samples in all packaging conditions investigated.

Figure 47.


Varying Dose Treatment, APC
Patties Stored 12 and 20 Days at 2 C
CFU/g
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
0
1
2
3
4
5
Gamma Dose (kGy)
Sgl Bag, 5% O2,D12
Sgl Bag, 21% O2,D12
Sgl Bag, 5% O2,D20
Sgl Bag, 21% O2,D20
Air-Pac, 21% O2,D20
Dual Bag,21% O2,D20

5.4.2 Microbiology

Patties were evaluated on days 0, 2, 4, 6, 8, 10, 13, 14, 16, 19, and 24 of storage (unirradiated control patties were only evaluated up to day 13). The samples were evaluated for APC, LAB, Y&M, and PSY.

The average (dual and single bag packaged) initial APC, LAB(Disk), LAB(Total), Y&M, and PSY assayed in unirradiated-control fresh ground beef on day 0 was approximately $1x10^7$/g, $5x10^5$/g, $2x10^6$/g, $1x10^5$/g, and $7x10^7$/g, respectively (Table 22). The average initial patty pH was about 6.0. There was virtually no difference between the number of organisms monitored in dual bag or single bag packaging by any assay, except for the Y&M assay. There were $7.9x10^4$/g and $1.3x10^5$/g Y&M present in dual and single bag packaging, respectively. The higher number present in the single bag package on day 0 may be due to the fact there was approximately a 12-hour delay between dual bag packaging and irradiation (storage time = 0), and a further 12 hour delay stored in a single bag before samples were analyzed. This 24-hour total delay at 4°C would allow for this amount of increase in Y&M.

Patties subjected to a 2.4 kGy (electron beam) irradiation experienced reductions in all groups of organisms monitored as compared to corresponding unirradiated control samples. Irradiated samples (dual and single bag) had average Log10 reductions of 3.9, 3.0, 3.6, 0.9, and 3.8 in APC, LAB(Disk), LAB(Total), Y&M and PSY, to an average of $1x10^3$/g, $5x10^2$/g, $5x10^2$/g, $1x10^4$/g and $1x10^4$/g, respectively (Table 22).

These Log10 reductions were comparable to those found when irradiating Fresh Ground Beef in Test #1 and #2 (sections 5.3.2 and 5.3.3). The reductions in these two tests were: APC 2.7 and 3.1, LAB(Disk) 2.8 and 3.0, and Y&M 0.8 for test #2 only.

Table 22. Refrigerated Storage, 4°C Fresh Ground Beef Irradiated and Control, Day 0 of Storage

Dose (kGy)	0	0	2.5	2.5	Log10 Reduction
Package	Dual	Single	Dual	Single	Dual/Sngl
pH	5.97	6.02	5.89	5.91	
			---CFU/g---		
APC	$1.0x10^7$	$1.2x10^7$	$1.2x10^3$	$1.2x10^3$	3.9/4.0
LAB (Disk)	$4.8x10^5$	$5.2x10^5$	$5.0x10^2$E	$4.7x10^2$E	3.0/3.0
LAB(Total)	$2.2x10^6$	$2.4x10^6$	$5.0x10^2$E	$4.7x10^2$E	3.6/3.7
Y&M	$7.9x10^4$	$1.3x10^5$	$9.6x10^3$	$1.2x10^4$	0.9/1.0
PSY	$6.9x10^7$	$7.0x10^7$	$1.1x10^4$	$1.4x10^4$	3.8/3.7

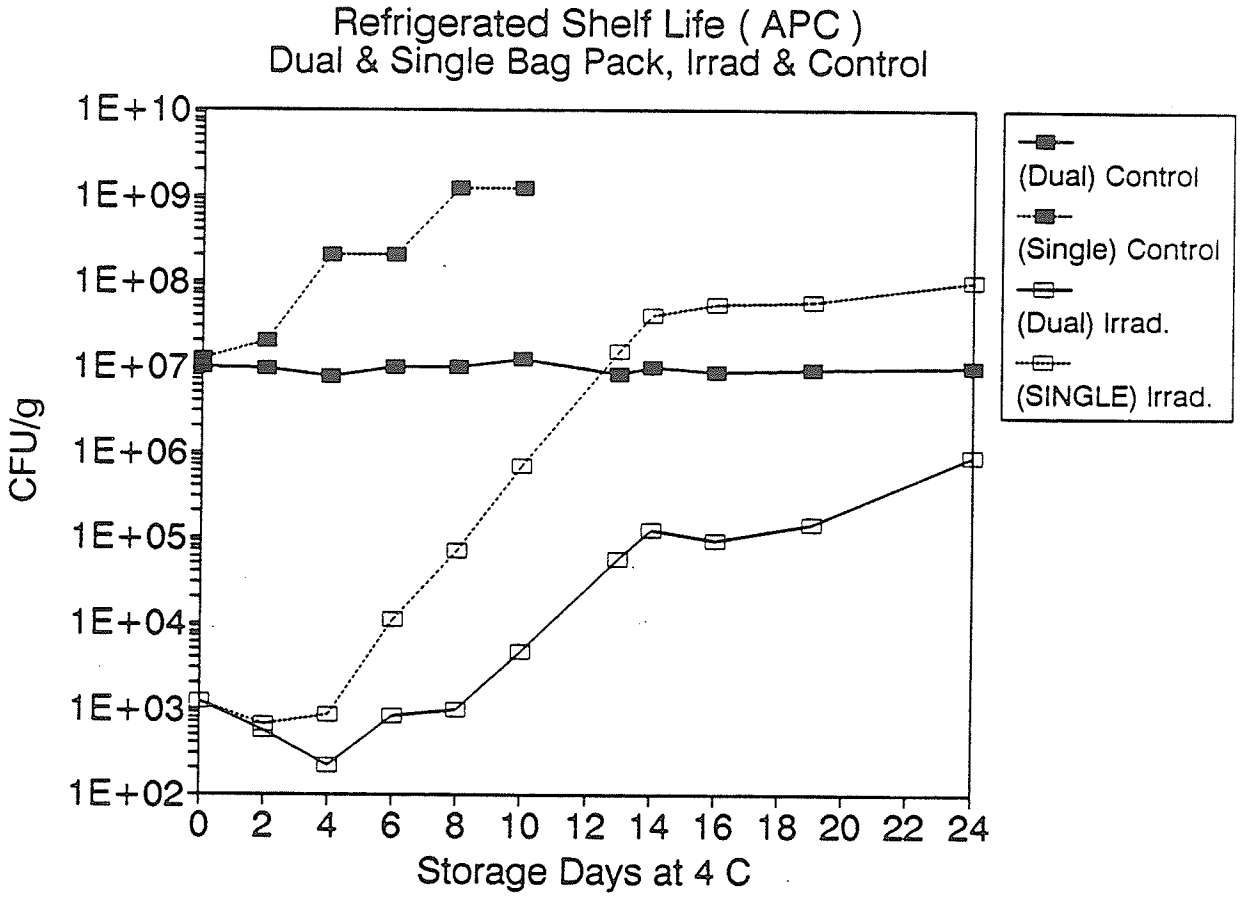
The APC of irradiated (2.38 kGy) patties and unirradiated control patties stored in dual and single bag packaging at 4°C are shown in Fig. 48. The APC of irradiated patties in both packaging conditions on day 0 of storage was approximately $1x10^3$/g. This is approximately a 4 Log10 reduction from the $1x10^7$/g in unirradiated control patties at this time (Fig. 48 and Table 22). The APC of irradiated patties continues to decline in patties stored in both dual and single bag packaging over the first 4 and 2 days of storage, respectively. The APC at these minimum levels for patties stored in dual and single bag packages

were an estimated $2.2x10^2$/g and $6.6x10^2$/g, respectively. Between day 4 and day 14 of storage, patties showed rapid increases of approximately 2.5 and 4.5 Log10 reaching $1.2x10^5$/g and $4.0x10^7$/g in dual and single bag packages, respectively. Between days 14 and 24, following the rapid growth phase there was a plateau were the APC was maintained with only a modest increase. The dual and single bag packaged patties reached $8.8x10^5$/g and $9.7x10^7$/g, respectively by day 24. The $5x10^7$/g APC spoilage criteria of Jay and Shelf (1978) was reached between days 14 and 16 for single bag packaged samples. The APC stayed at this spoilage level until day 19. The dual bag packaged samples had not reached the spoilage level by day 24 of storage. Thus the microbiological shelf life was approximately 16 days for single bag packaged samples and >24 days for dual bag packaged samples.

These results agree with the preliminary results found in the raw ground beef test #2 with storage at 4°C (section 5.3.4.3), where the microbiological spoilage level was reached in 15 days and >21 days for irradiated dual and single bag packaged samples, respectively. The APC for patties in both packaging conditions decreased between days 15 and 21 in the preliminary experiment (Fig. 41), while they increased slightly in this experiment.

The APC of unirradiated control patties stored at 4°C in dual and single bag packaging are shown in Fig. 48. The initial APC on day 0 of patties in either packaging condition was approximately $1.0x10^7$/g (Fig. 48 and Table 22).

Figure 48.


Refrigerated Shelf Life (APC)
Dual & Single Bag Pack, Irrad & Control
CFU/g
1E+10
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
0 2 4 6 8 10 12 14 16 18 20 22 24
Storage Days at 4 C
(Dual) Control
(Single) Control
(Dual) Irrad.
(SINGLE) Irrad.

The APC of the dual bag packaged patties did not increase over the 24 days of storage. It remained at approximately $1.0x10^7$/g. Thus the microbiological spoilage level of $5x10^7$/g was not reached by day 24 of storage. This result differed from the findings in raw ground beef test #2 stored at 4°C (section 5.3.4.3), where the spoilage level was reached by approximately day 12. In that experiment the APC increased from approximately $4x10^6$/g on day 0 to $1x10^8$/g by day 15 followed by a decrease to $4x10^7$/g on day 21 (Fig. 41).

The APC of the single bag packaged, unirradiated control patties increased rapidly upon storage at 4°C (Fig. 48). The APC increased from $1.2x10^7$/g on day 0 to $2.0x10^8$/g on day 8. The microbiological spoilage level was reached on approximately day 3 of storage. The APC increased to $>1x10^9$/g by day 8. A 3-day microbiological shelf life was also found in the raw ground beef test #2 (section 5.3.4.3); however. the APC only reached a high of $1x10^8$/g by day 15 (Fig. 41).

5.4.2.1 Lactic Acid Method

The LAB in irradiated dual and single bag packaged patties stored at 4°C were evaluated using pour plates of MRS media incubated at 35° and 20°C. The total number of typical dot and disk shaped colonies (LAB-Total), and the number of disk shaped colonies only (LAB-Disk), were recorded. In previous experiments the LAB was given as the number of disk shaped colonies only, when incubated at 35°C. There was some concern that a high incubation

temperature (35°C) would not allow for proper monitoring of the LAB group, so a 20°C incubation temperature was included.

The LAB results for irradiated patties stored in dual bag packaging at 4°C monitored using both incubation temperatures and recording the number of total and disk only colonies are shown in Fig. 49. The Y&M assay results are also included in this figure. The results for total and disk only colonies when plates were incubated at 35°C were almost identical, except for the day 13 and day 14 samples where the total colonies were approximately 1.5 and 1 Log10 greater than the disk only value for LAB. When plates are incubated at 20°C the number of total and disk colonies observed is greater than the corresponding number when incubated at 35°C. The trend observed for MRS-20°C, Disk only is consistent with those shown by MRS-35°C, Disk Only and MRS-35°C, Total. The MRS-20°C, Total results were greater than the other assays and were comparable to the results found for Y&M. This fact, plus the observation of "fuzzy" shaped colonies in the 20°C incubated assay leads to the conclusion that possibly some of the dot shaped colonies that are contributing to the total count may indeed be small fuzzy Y&M colonies.

The LAB results for irradiated patties stored in single bag packaging at 4°C monitored using both incubation temperatures and recording the number of total and disk only colonies are shown in Fig. 50. The results for the number of total and disk only colonies were identical, except for between days 8 and 16 of storage. The number of total colonies were approximately 2 Log10 greater than

the disk only values during this period. This period was also when the Y&M assay experienced rapid growth, possibly causing interference with this assay. When plates were incubated at 20°C the number of disk only colonies were identical to the corresponding number of disk only colonies when incubated at 35°C between days 13 to 24. Prior to day 13 the number of disk only colonies were greater when plates were incubated at 20°C as compared to 35°C. The number of total colonies when plates were incubated at 20°C were almost identical to total colonies when incubated at 35°C except between day 8 to 16 of storage, as was found with corresponding 35°C incubated plates. The number of total colonies monitored during this period were almost identical to the number of Y&M. The rapid growth of Y&M during this day 8 to 16 period may be causing interference with the total colony count in this assay and the 35°C incubated assay. As stated above, the Y&M may be interfering with the number of total colonies as most of the "dot" colonies contributing to the total count may be in fact small fuzzy colonies.

The LAB results for unirradiated dual bag packaged patties stored at 4°C are shown in Fig. 51. The number of disk only colonies on 35°C incubated plates increases gradually from approximately $5x10^5$/g to $2x10^6$/g over the 24-day storage period. The number of total colonies from plates incubated at 35°C are between 0.1 and 0.7 Log10 greater than the number of disk only colonies (Fig. 51). There are approximately 1.5 Log10 more disk only colonies on 20°C incubated plates as compared to 35°C incubated plates. A similar trend is

observed when comparing the total number of colonies on plates incubated at the two temperatures. There appears to be no interference with any of the assays by Y&M. This appears to be due to the fact the number of Y&M is generally less than the number of LAB as recorded on all of the assays (Fig. 51).

The LAB results for unirradiated single bag packaged patties stored at 4°C are shown in Fig. 52. The results in this packaging condition were similar to those found in dual bag packaged patties with respect to the relationship between the two shapes of organisms recorded using the two plate incubation temperatures.

The MRS-Disk only results will be used to represent the LAB group of organisms. The use of total "typical" colonies (dot and disc) was shown to be affected by the yeast and mould if present in larger numbers than the disc only colonies. This problem was especially prevalent in assays with a plate incubation temperature of 20°C. Although the use of a 20°C incubation temperature as compared to 35°C yields >1 Log10 more disc only shaped colonies, the growth trends demonstrated by both are similar.

5.4.2 Microbiology (Continued)

The LAB results (35°C, 3-day incubated plates and recording Disc only shaped colonies) for irradiated (2.4 kGy) patties and unirradiated control patties stored in dual and single bag packaging at 4°C are shown in Fig. 53. The LAB of irradiated patties in both packaging conditions on day 0 of storage was

estimated to be approximately $5x10^2$/g. This is a 3 Log10 reduction from the $5x10^5$/g in unirradiated control patties at this time (Fig. 53 and Table 22). The LAB of irradiated patties continues to decline in patties stored in both dual and single bag packaging over the first 4 and 6 days of storage, respectively. The LAB at these minimum levels for patties stored in dual and single bag packages were an estimated $6.0x10^1$/g and $4.5x10^1$/g, respectively. Between these low points and day 16 of storage, patties showed rapid increases of approximately 2.5 and 4.0 Log10 reaching $1.6x10^4$/g and $6.5x10^5$/g in dual and single bag packages, respectively. Between days 16 and 24 growth was more gradual while dual bag packaged patties maintained a LAB level approximately 1.5 Log10 lower than the corresponding single bag packaged patty, reaching $5.0x10^5$/g and $1.5x10^7$/g on day 24 of storage.

The LAB results for unirradiated control patties stored in dual and single bag packaging are shown in Fig. 53. The LAB unirradiated control patties in both packaging conditions on day 0 of storage was on average $5.0x10^5$/g (Table 22). The LAB of dual bag packaged patties increased gradually to $3.0x10^6$/g by day 24 of storage - less than a 0.8 Log10 increase over the 24-day storage period. The LAB of the single bag packaged patty increased to $5.0x10^6$/g by day 4 and reached and maintained $1.3x10^7$/g by day 10 to 13 of storage. Between days 6 and 13 of storage the LAB of single bag packaged patties were approximately 1 Log10 greater than the dual bag packaged patties.

Figure 49.

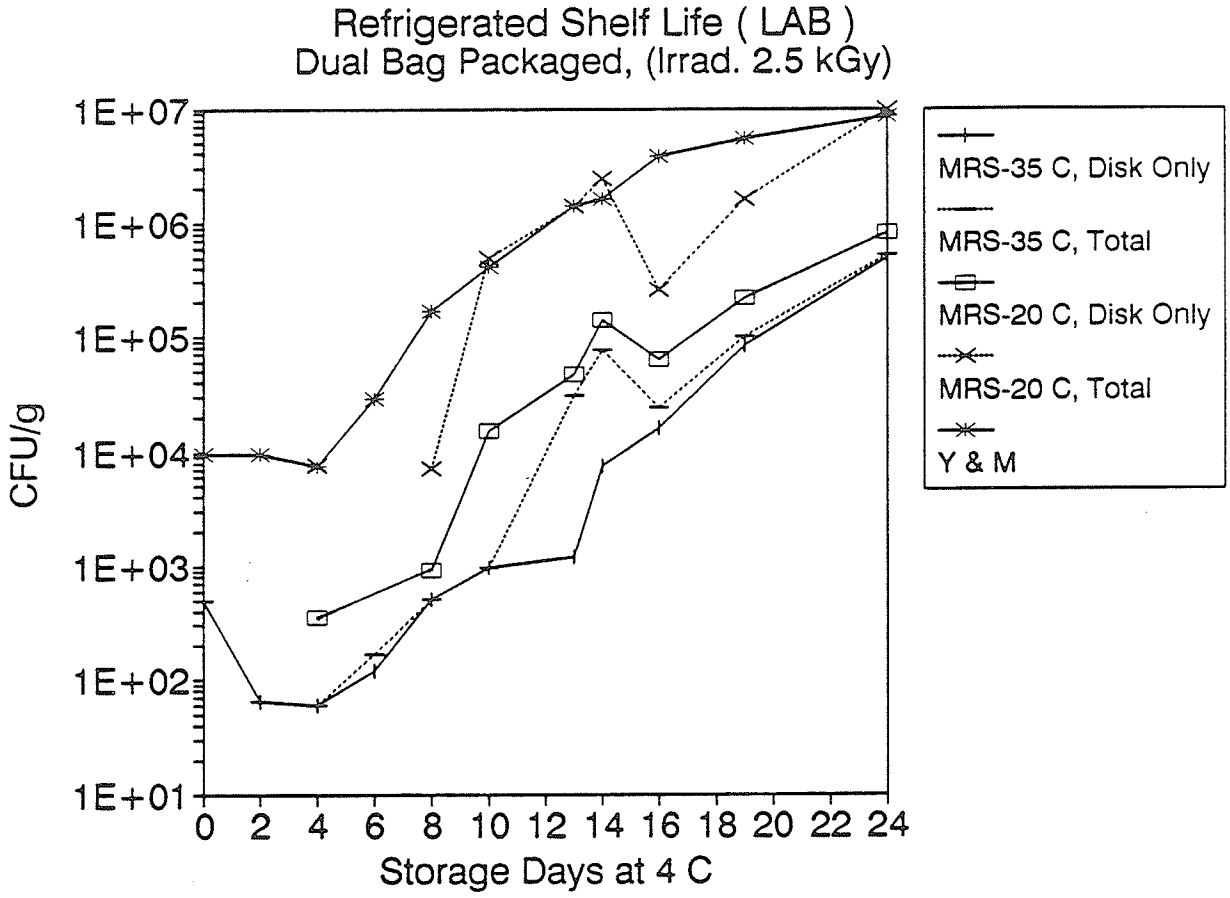

Refrigerated Shelf Life (LAB)
Dual Bag Packaged, (Irrad. 2.5 kGy)
CFU/g
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
0 2 4 6 8 10 12 14 16 18 20 22 24
Storage Days at 4 C
MRS-35 C, Disk Only
MRS-35 C, Total
MRS-20 C, Disk Only
MRS-20 C, Total
Y & M

Figure 50.

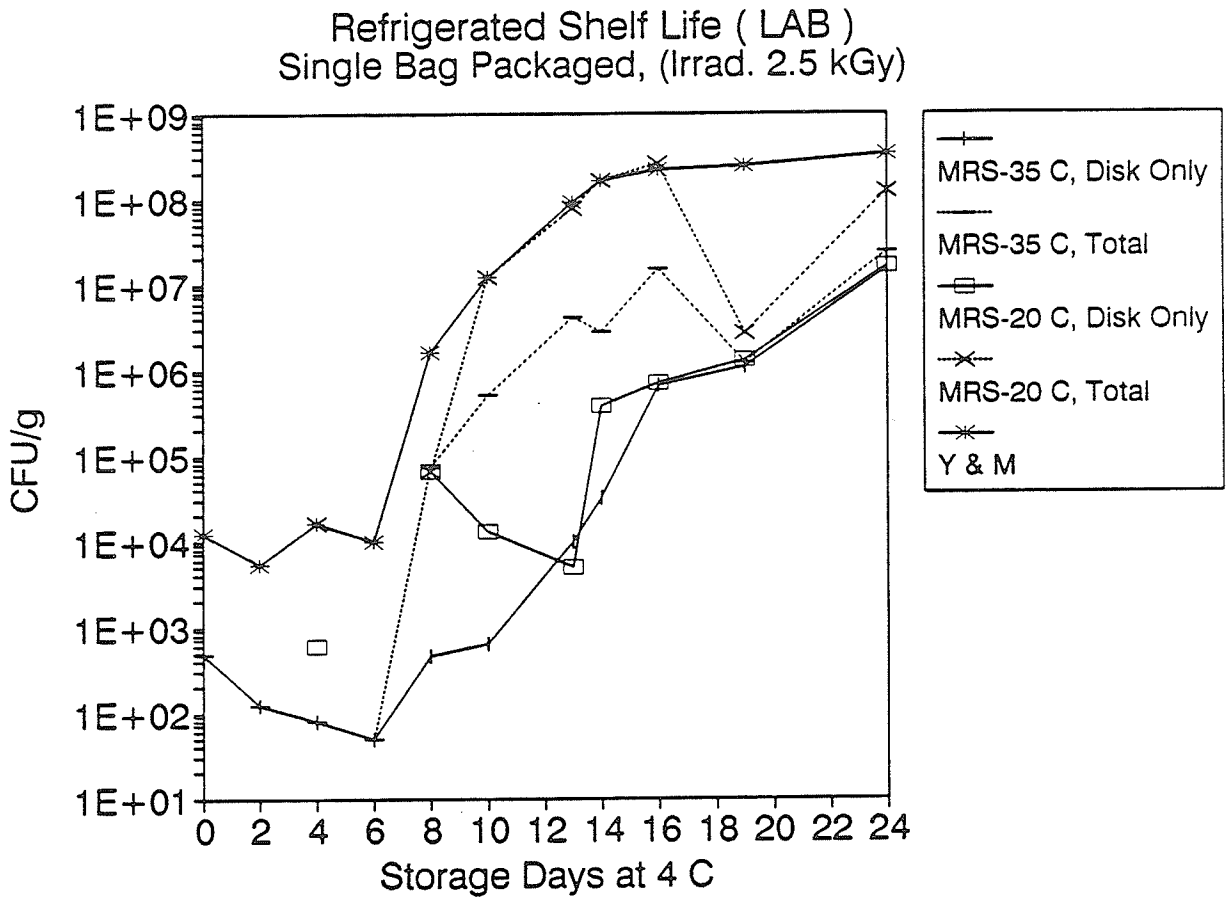

Refrigerated Shelf Life (LAB)
Single Bag Packaged, (Irrad. 2.5 kGy)
CFU/g
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
0 2 4 6 8 10 12 14 16 18 20 22 24
Storage Days at 4 C
MRS-35 C, Disk Only
MRS-35 C, Total
MRS-20 C, Disk Only
MRS-20 C, Total
Y & M

Figure 51.

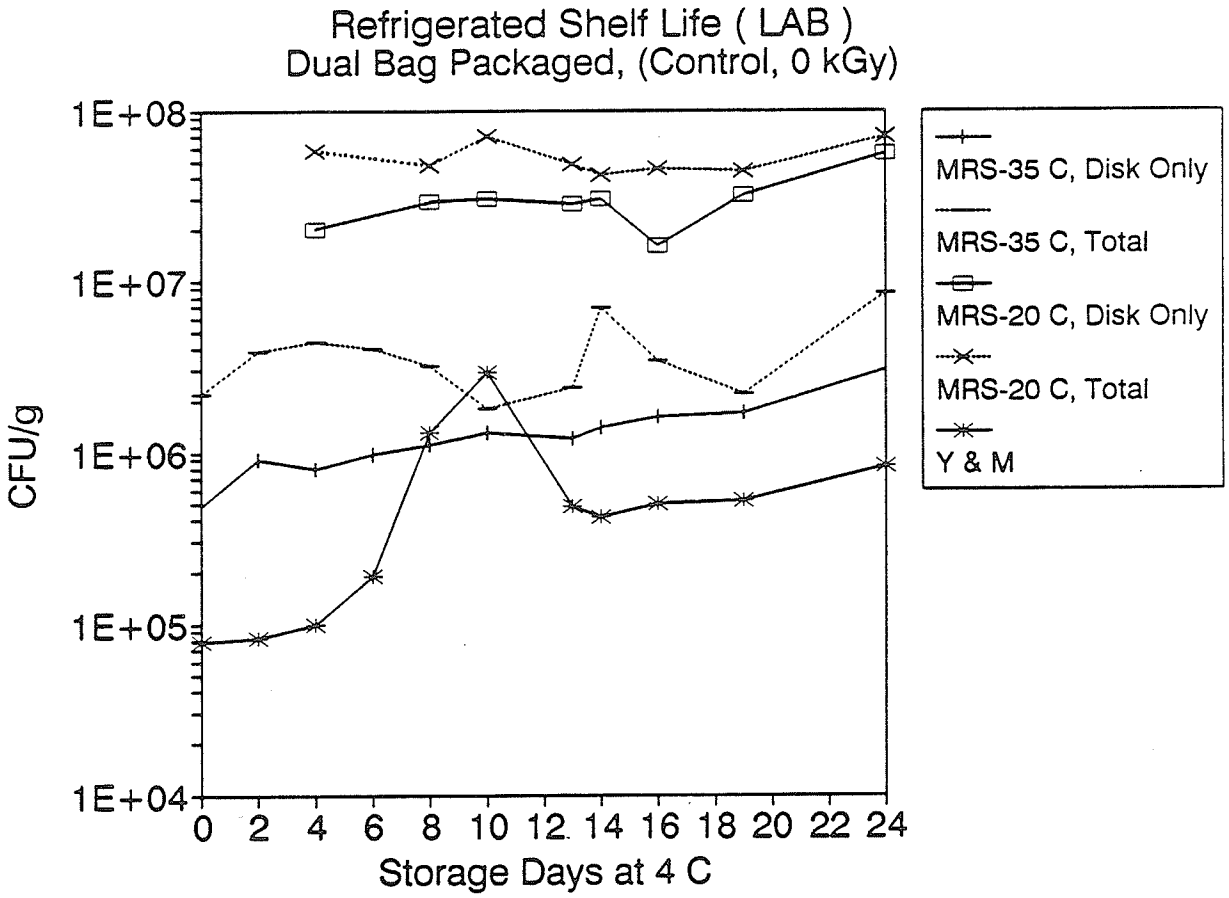
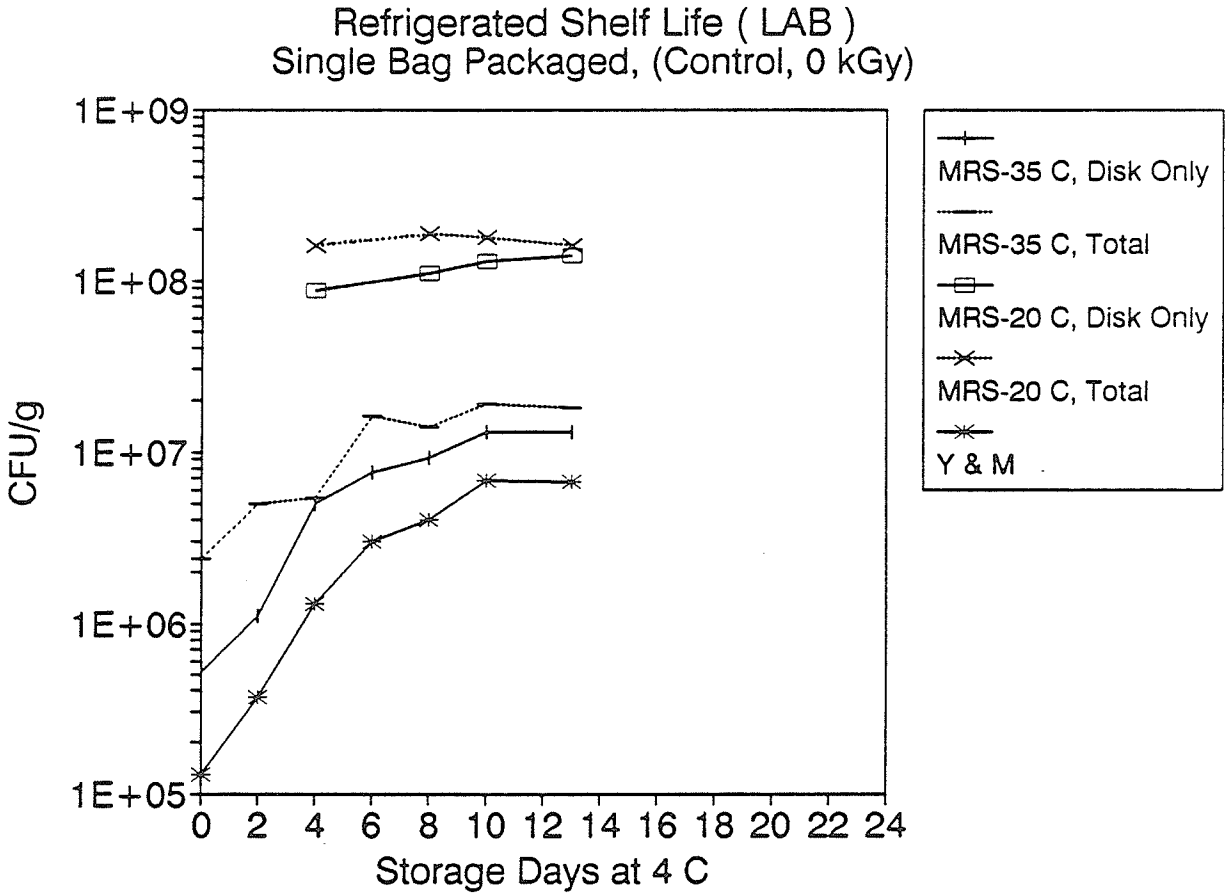

Refrigerated Shelf Life (LAB)
Dual Bag Packaged, (Control, 0 kGy)
CFU/g
1E+08
1E+07
1E+06
1E+05
1E+04
0 2 4 6 8 10 12 14 16 18 20 22 24
Storage Days at 4 C
MRS-35 C, Disk Only
MRS-35 C, Total
MRS-20 C, Disk Only
MRS-20 C, Total
Y & M

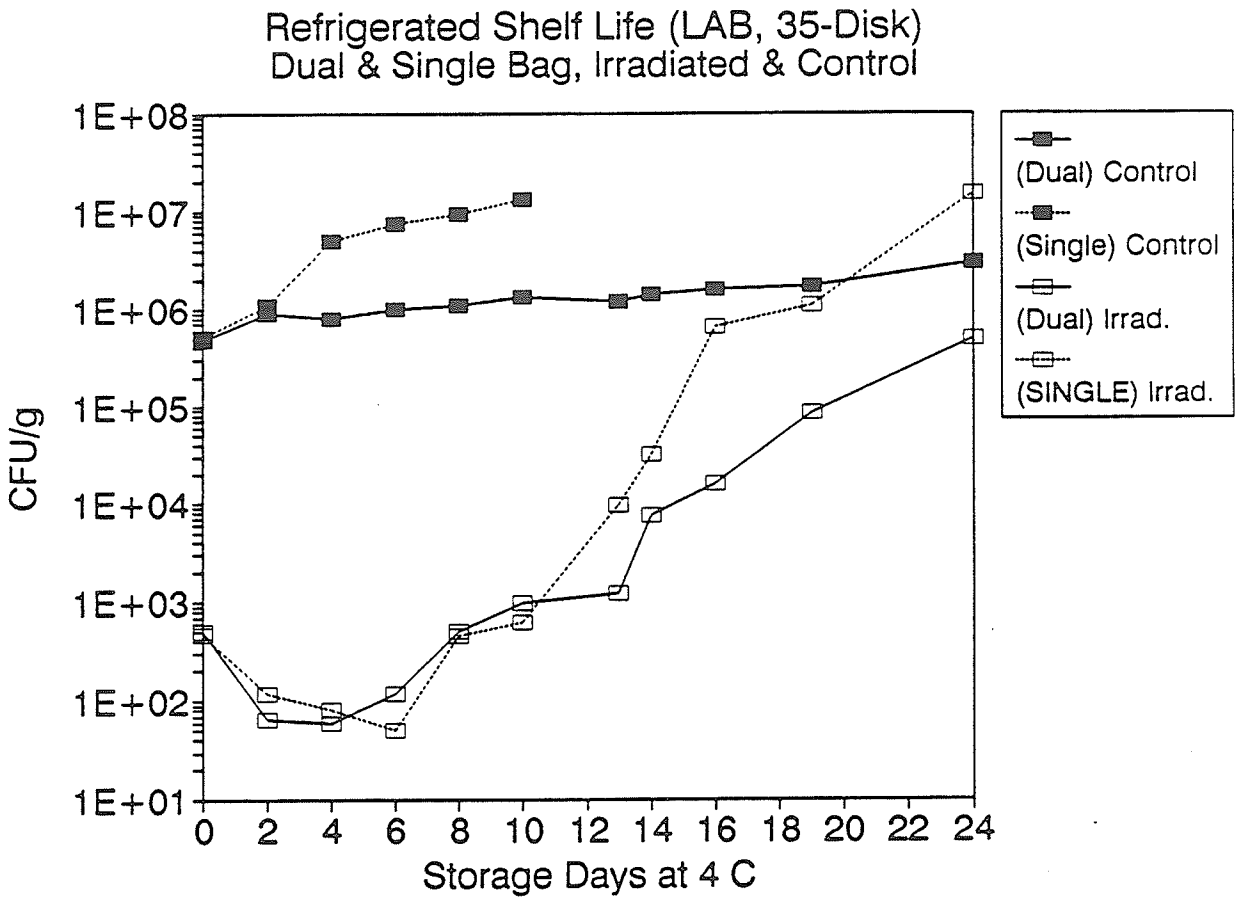
Figure 52.


Refrigerated Shelf Life (LAB)
Single Bag Packaged, (Control, 0 kGy)
CFU/g
1E+09
1E+08
1E+07
1E+06
1E+05
0 2 4 6 8 10 12 14 16 18 20 22 24
Storage Days at 4 C
MRS-35 C, Disk Only
MRS-35 C, Total
MRS-20 C, Disk Only
MRS-20 C, Total
Y & M

The Y&M in irradiated (2.4 kGy) patties and unirradiated control patties stored in dual and single bag packaging at 4°C are shown in Fig. 54. The Y&M of irradiated patties in both packaging conditions on day 0 of storage was approximately $1x10^4$/g. This is approximately a 1 Log10 reduction from the $1x10^5$/g in unirradiated control patties at this time (Fig. 54 and Table 22). The Y&M of irradiated patties was maintained at this level in patties stored in both dual and single bag packaging over the first 4 and 6 days of storage, respectively. The Y&M between these points and day 14 of storage patties showed rapid increases of approximately 2.0 and 4.0 Log10 reaching $1.6x10^6$/g and $1.6x10^8$/g in dual and single bag packages, respectively. Between days 14 and 24, growth was more gradual while dual bag packaged patties maintained an Y&M level approximately 1.5 Log10 lower than the corresponding single bag packaged patties, reaching $8.6x10^6$/g and $3.3x10^8$/g on day 24 of storage.

The Y&M results for unirradiated control patties stored in dual and single bag packaging are shown in Fig. 54. The average Y&M for patties stored in both packaging conditions was approximately $1x10^5$/g on day 0 (Fig. 54 and Table 22). The variation between dual and single bag packaging can be explained by the time delay in actually performing the microbiological evaluation (as described above). If the single bag packaged line were extrapolated to 24 hours (the estimated time delay in evaluation) the Y&M value would be close to the $7.9x10^4$/g value for dual bag packages as opposed to the $1.3x10^5$/g value recorded as day 0 of storage (Table 22).

Figure 53.


Refrigerated Shelf Life (LAB, 35-Disk)
Dual & Single Bag, Irradiated & Control
CFU/g
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
1E+01
0 2 4 6 8 10 12 14 16 18 20 22 24
Storage Days at 4 C
(Dual) Control
(Single) Control
(Dual) Irrad.
(SINGLE) Irrad.

The Y&M of unirradiated dual bag packaged patties increased gradually to $8.3x10^5$/g by day 24 (Fig. 54). This is less than a 1 Log10 increase over 24 days. There was a substantial deviation for this trend from samples evaluated on day 8 and day 10 of storage. The duplicate samples evaluated on day 8 and day 10 had wide variations in Y&M evaluated between duplicates. The wide range between duplicates was not, however, observed in the APC, LAB, and PSY assays. If the value for the sample reading high was neglected, the lower value would fall on the gradual increasing line of Y&M for dual bag samples. The higher values appear to fall upon the single bag packaged line. This could have be caused by one of the duplicates dual bag packaged samples for days 8 and 10 having a leak in the outer bag (rendering the sample similar to a single bag sample). This theory is not consistent with the similarity in values obtained between duplicate samples for the other microbiological assays. Contamination of the higher Y&M valued duplicate during plating may also be a reason for the high Y&M counts observed.

The Y&M of unirradiated control single bag packaged patties increased rapidly from $1.3x10^5$/g on day 0 to $3.0x10^6$/g on day 6 (Fig. 54 and Table 22). This was a 1.4 Log10 increase over 6 days in storage. Between day 6 and day 13 of storage there was only a further 0.35 Log10 increase in Y&M.

The Y&M group of organisms is the only group monitored that resulted in higher numbers of organism upon storage at 4°C after irradiation as opposed to no irradiation. If patties were stored for longer than approximately 10 days

at 4°C in either packaging condition, there would be more Y&M present in irradiated samples as compared to unirradiated control samples. This selection for Y&M in 4°C stored patties in either packaging condition is a result of the Y&M having a larger radiation D10 than the other groups of organisms. This lower reduction from the initial number of organisms due to irradiation means there are more Y&M present on day 0 than other groups of organisms in irradiated patties. This reduction in the competitive microflora along with the 4°C storage temperature also selects for Y&M. We have shown the Y&M remaining in irradiated patties are psychrotrophic (section 5.3.4.3) and in this experiment, thus they can grow at refrigerated temperatures.

The PSY in irradiated (2.4 kGy) patties and unirradiated control patties stored in dual and single bag packaging at 4°C are shown in Fig. 55. The PSY of irradiated patties in both packaging conditions on day 0 of storage was approximately $1x10^4$/g. This is approximately a 3.8 Log10 reduction from the $7x10^7$/g in unirradiated control patties at this time (Fig. 55 and Table 22). The PSY of irradiated patties was maintained at this level in patties stored in both dual and single bag packaging over the first 4 and 2 days of storage, respectively. The Y&M between these points and day 14 of storage patties showed rapid increases of approximately 2.3 and 4.0 Log10 reaching $2.2x10^6$/g and $1.4x10^8$/g in dual and single bag packages, respectively. Between days 14 and 24 growth was more gradual while dual bag packaged patties maintained an PSY level

approximately 1.5 Log10 lower than the corresponding single bag packaged patties, reaching $2.1x10^7$/g and $6.3x10^8$/g on day 24 of storage.

The PSY and Y&M growth curves for irradiated (2.4 kGy) patties stored in dual and single bag packaging at 4°C are shown in Fig. 56. The PSY growth curves for dual and single bag packaged patties superimposes on the corresponding Y&M curves. This indicates that the organisms being monitored with the selective Y&M assay are the same as those being monitored on the more general Psy assay. Thus the psychrotrophic organisms monitored are in fact yeast and mould. This was confirmed by the shape and size of colonies on the PSY assay, which were typical of yeast and mould. There are more PSY and Y&M present in single bag packaged patties than dual bag packaged patties after a 6-day lag in growth. This suggests these organisms grow better in the presence of oxygen. This result agrees with the findings of raw ground beef stored at 4°C Test #2 (section 5.3.4.3).

The pH of irradiated and unirradiated control patties stored in both types of packaging conditions at 4°C are shown in Fig. 57. The patty pH on day 0 for irradiated and unirradiated control patties was approximately 6.0 and 5.9, respectively (Fig. 57 and Table 19). The unirradiated control patties were 0.1 higher than the starting patty pH of 5.9, found in raw ground beef Test #2 (section 5.3.4.3). The pH of the single bag packaged patties (irradiated and unirradiated control) increased to levels greater than the initial pH over the

storage periods monitored (Fig. 57). The patty pH of dual bag packaged patties (irradiated and unirradiated control) decreased and remained below the initial pH over the storage period monitored.

The patty pH of dual bag packaged irradiated patties decreased gradually from 5.9 on day 0 to 5.4 by day 24 of storage at 4°C (Fig. 57). These results agree with those found in section 5.3.4.3 (Fig. 46), where the pH decreased from the day 0 initial level and remained below this level during the 21-day storage period.

The patty pH of single bag packaged irradiated patties decreased gradually from 5.9 on day 0 to 5.8 by day 10 (Fig.57 and Table 22). Between day 10 and 24 of storage the pH increased rapidly to a level of 6.9 by day 24. This result is similar to that found in section 5.3.4.3. Patties subjected to similar treatments increased to a pH of 6.7 by day 21 of storage after remaining at the initial pH until day 7 (Fig. 46).

The pH of unirradiated control patties stored in dual bag packaging decreased rapidly from 6.0 on day 0 to 5.5 by day 2. The pH remained at this level until day 6. Between day 6 and day 24 there was a gradual increase to pH 5.8 by day 24. This day 24 pH was still lower than the initial day 0 pH. These results are not the same as results found in the raw ground beef Test #2 (section 5.3.4.3). Patties with a similar treatment showed increases in pH (Fig. 46) from the initial day 0 level, on days 7, 15, and 21 of storage at 4°C.

Figure 54.

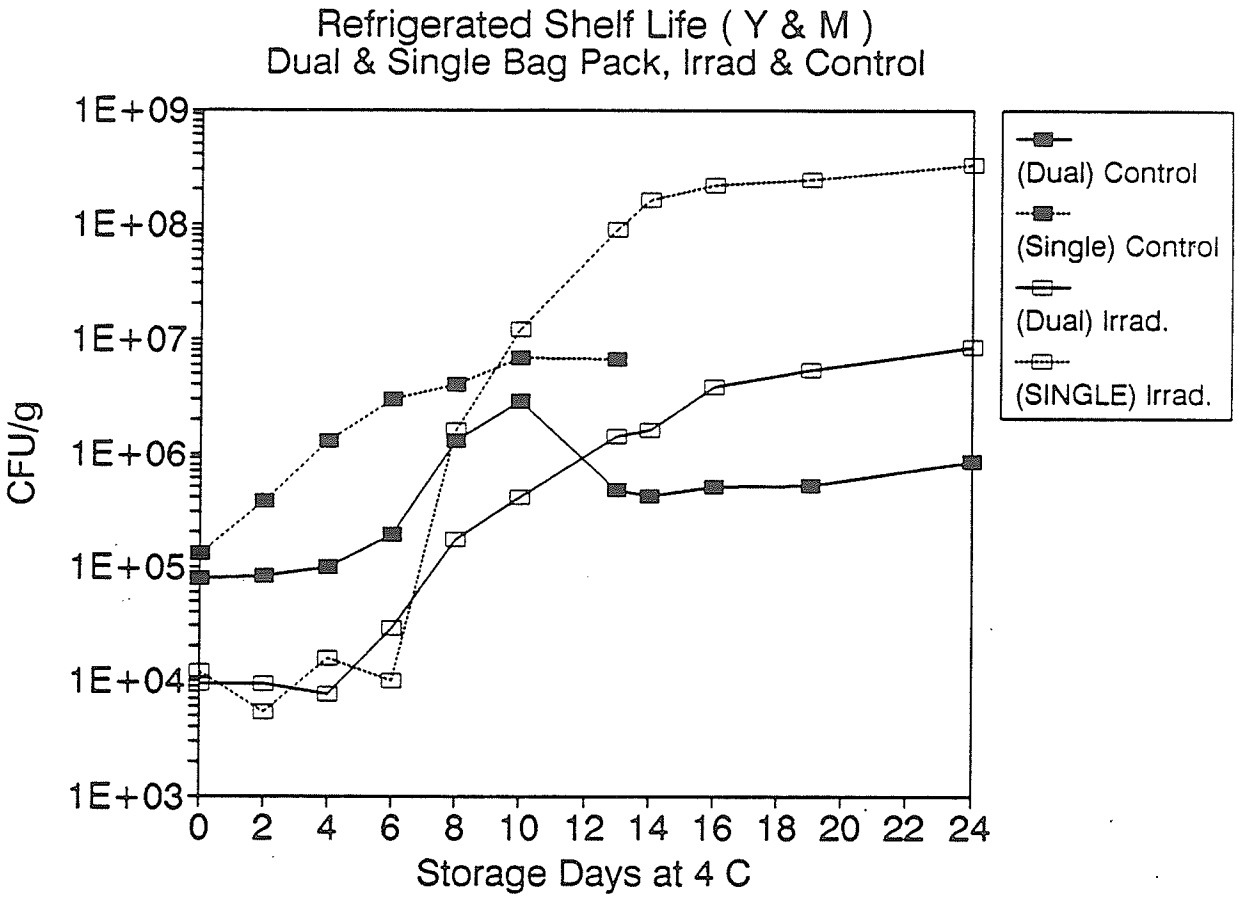

Refrigerated Shelf Life (Y & M)
Dual & Single Bag Pack, Irrad & Control
CFU/g
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
0 2 4 6 8 10 12 14 16 18 20 22 24
Storage Days at 4 C
(Dual) Control
(Single) Control
(Dual) Irrad.
(SINGLE) Irrad.

Figure 55.

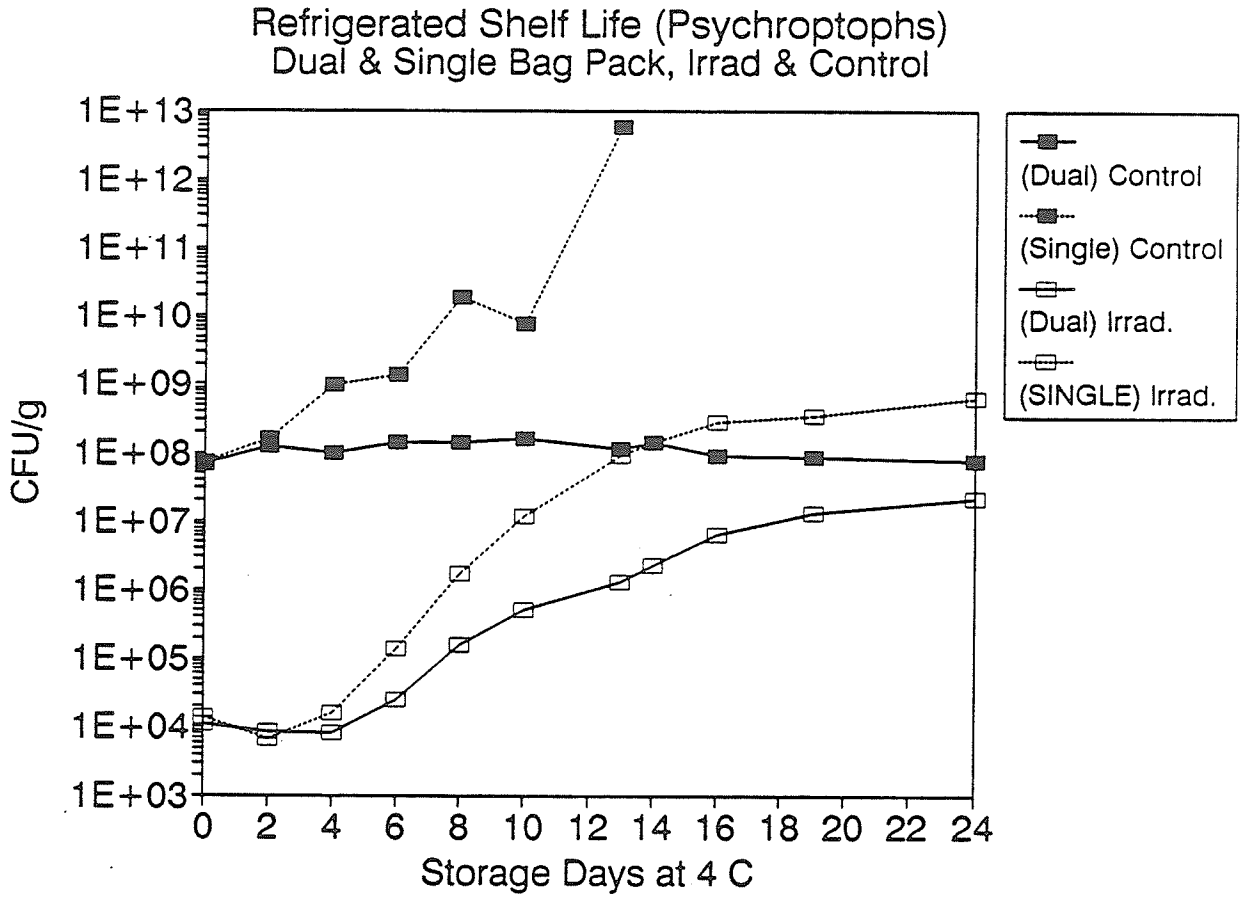

Refrigerated Shelf Life (Psychroptophs)
Dual & Single Bag Pack, Irrad & Control
CFU/g
1E+13
1E+12
1E+11
1E+10
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
0 2 4 6 8 10 12 14 16 18 20 22 24
Storage Days at 4 C
(Dual) Control
(Single) Control
(Dual) Irrad.
(SINGLE) Irrad.

Figure 56.

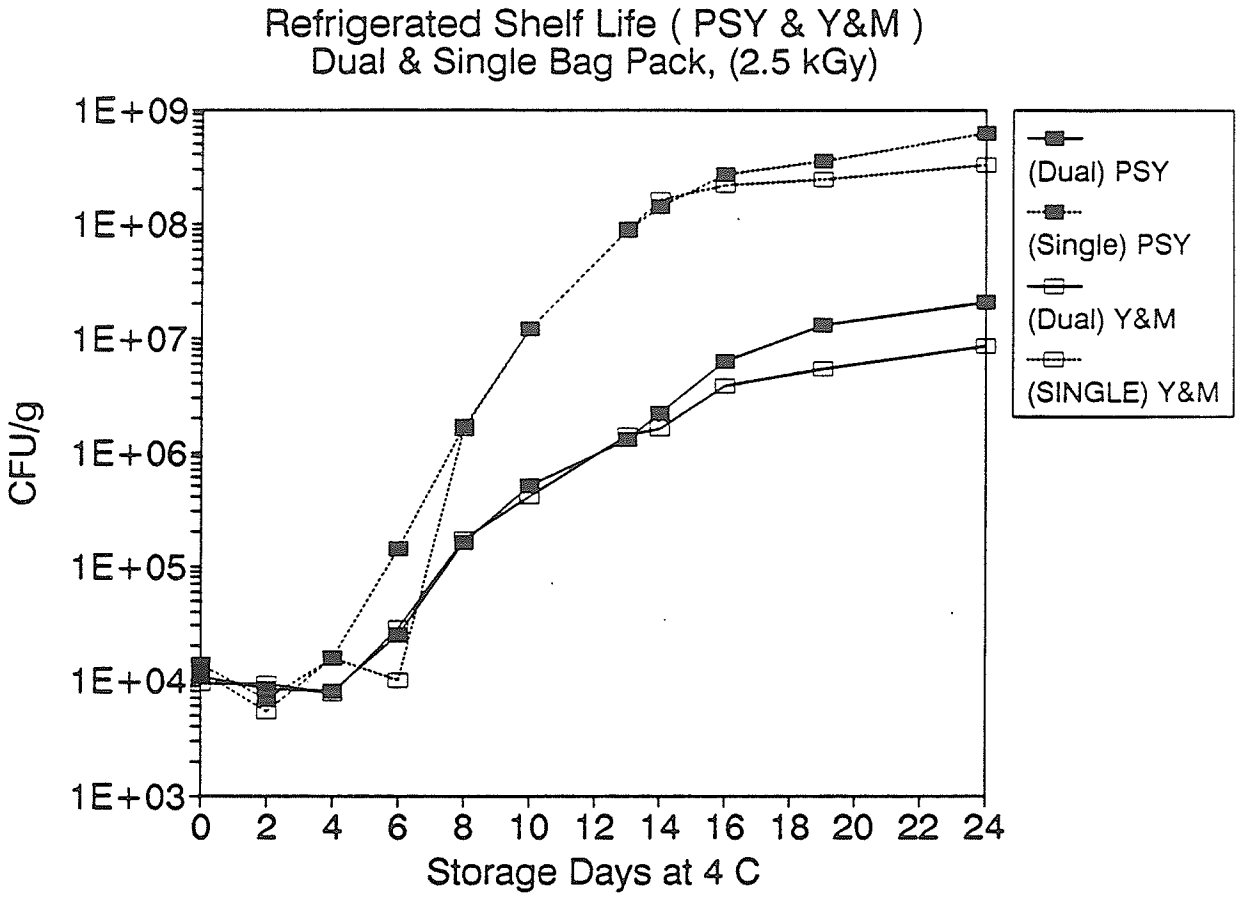
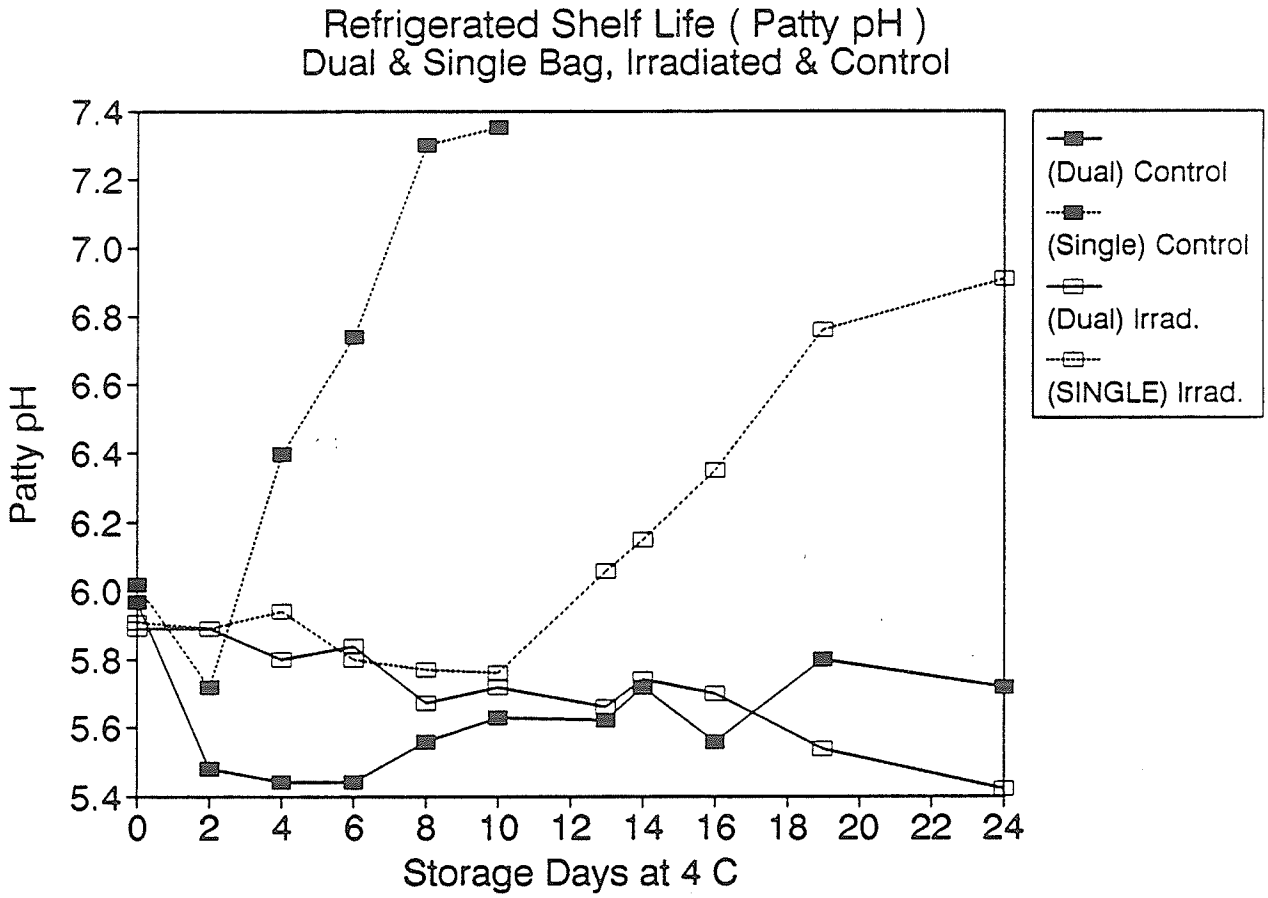

Refrigerated Shelf Life (PSY & Y&M)
Dual & Single Bag Pack, (2.5 kGy)
CFU/g
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
0 2 4 6 8 10 12 14 16 18 20 22 24
Storage Days at 4 C
(Dual) PSY
(Single) PSY
(Dual) Y&M
(SINGLE) Y&M

Figure 57.


Refrigerated Shelf Life (Patty pH)
Dual & Single Bag, Irradiated & Control
Patty pH
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
0
2
4
6
8
10
12
14
16
18
20
22
24
Storage Days at 4 C
(Dual) Control
(Single) Control
(Dual) Irrad.
(SINGLE) Irrad.

The pH of unirradiated control patties stored in single bag packaging decreased rapidly from 6.0 on day 0 to 5.7 by day 2 (Fig. 57). Between day 2 and day 10 of storage the pH increased rapidly reaching 7.4 by day 10. This result was also found in the raw ground beef Test #2 (section 5.3.4.3) where the pH reached 7.4 by day 15 of storage (Fig. 46).

5.4.3 Colour

The colour of the patties was monitored throughout the storage period. Patty colour is an important attribute used by a consumer to determine if hamburger is acceptable. Patty colour was monitored subjectively by visual observation, and objectively using a Hunter Colour Difference Meter.

5.4.3.1 Visual Colour Monitoring

The subjective term used to describe the colour of patties at day 0 of storage was dull red. The irradiated and unirradiated control patties stored in dual or single bag packaging at 4°C all were described as dull brown by day 2 of storage (Table 23). A visually appealing patty colour was not observed after day 0 of storage. As storage time increased the amount of redness observed on patties decreased (Table 23). This was expected as it is well known that nitrogen packaged meats have poor red colour during refrigerated storage. Surface slime was observed sooner on patties stored in single bag packaging than corresponding irradiated or unirradiated control dual bag packaged patties.

Slime was observed on irradiated and unirradiated control patties stored in single bag packaging on day 19 and 8, respectively. Surface slime was not observed on any irradiated or unirradiated control dual bag packaged patties during the storage period investigated.

Table 23. Patty Colour, Visual Observation, Storage at 4°C

Dose	0 kGy	0 kGy	2.4 kGy	2.4 kGy
Pack	Dual	Single	Dual	Single
Day				
0	Dull Red	Dull Red	Dull Red	Dull Red
2	Dull Brown	Dull Brown	Dull Brown	Dull Brown
4	Sandy Brown*1	Sandy Brown	Sandy Brown*1	Sandy Brown
6	Sandy Brown	Sandy Brown*2	Sandy Brown	Sandy Brown
8	Light Brown	Brown (Slime)	Light Brown	Light Brown
10	Dull Brown	Red (Slime)	Sandy Brown	Sandy Brown
13	Dull Brown	Red (Slime)	Sandy Brown	Sandy Brown*3
14	Dull Brown		Sandy Brown	Sandy Brown*3
16	Dull Brown		Dull Brown	Sandy Brown*3
19	Dull Brown		Dull Brown	Brown (Slime)
21	Dull Brown*4		Dull Brown	Brown (Slime)

*1 Greyish
*2 Red Tinge
*3 Some Dark Brown Spots
*4 Some Dark Green Spots

5.4.3.2 Hunterlab Colour Difference Meter

The Hunter L, A, and B values for all patties were measured through the D-955 inner bag using a HunterLab Colour Difference Meter. Calibration with

a black and white standard tile was performed with D-955 film covering the calibration tiles for consistency when measuring patties. The Hunter A value which measures the degree of redness was evaluated over the storage period (Fig. 58). The Hunter A value for fresh full bloom ground beef was measured at 13.9.

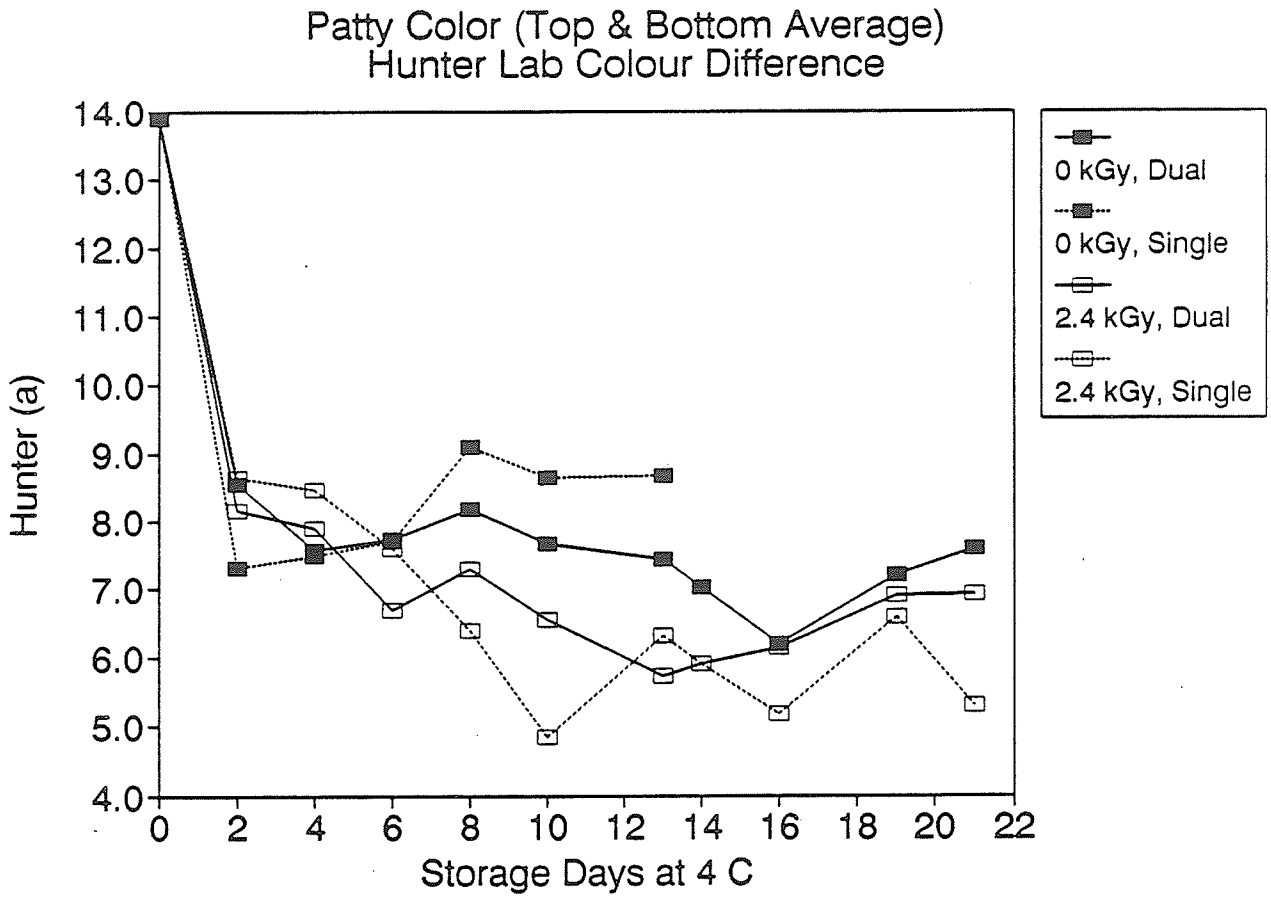
Unirradiated Control: The Hunter A value of unirradiated patties stored in dual and single bag packaging dropped to approximately 8.5 and 7.5, respectively by day 2 of storage (Fig 58). This reduction of redness agrees with the visual dull brown observations (Table 23). The Hunter A value for both the dual and single bag packaged patties were approximately 7.5 between day 4 and 6. There were slight increases on day 19 and day 8 for dual and single bag packaged patties, respectively. These slight increases at later stages of storage were minimal and the visual patty colour observed would not be acceptable to consumers.

Irradiated: The Hunter A value of 2.4 kGy irradiated patties stored in dual and single bag packaging were both approximately 8.5 (Fig 58). This was a substantial decrease from the 13.9 Hunter A value for fresh full bloom ground beef and agrees with the dull brown observation (Table 23). Further storage of patties in either packaging condition generally resulted in decreased Hunter A

values. The Hunter A values remained lower than 7 (Fig. 58) and this agrees with the lack of redness visually observed on all patties after Day 0 of storage.

The poor colour (lack of redness) of unirradiated and irradiated patties in dual and single bag packaging at all stages of storage as indicated by visual observations (Table 23) and low Hunter A values (Fig. 58) were of concern. Shivas et Al. (1984) found that the addition of 0.01, 0.05, and 0.10% ascorbic acid to ground beef resulted in higher visual colour scores and CIE a* readings during refrigerated storage up to 10 days. The effect on visual colour observed and Hunter A value after the addition of crystalline ascorbic acid to ground beef at a level 0.01% was investigated.

Figure 58.


Patty Color (Top & Bottom Average)
Hunter Lab Colour Difference
Hunter (a)
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
0
2
4
6
8
10
12
14
16
18
20
22
Storage Days at 4 C
0 kGy, Dual
0 kGy, Single
2.4 kGy, Dual
2.4 kGy, Single

5.4.4 Odour

Table 24. Odour of Patties, Storage at 4°C

Dose	0 kGy	0 kGy	2.4 kGy	2.4 kGy
Pack	Dual	Single	Dual	Single
Day				
0	O.K.	O.K.	O.K.	O.K.
2	O.K.	O.K.	O.K.*1	O.K.
4	Slight Pungent	Off	O.K.	O.K.
6	Slight Pungent	Off	O.K.	O.K.
8	Slight Pungent	Foul	O.K.	O.K.
10	Pungent	Very Foul	O.K.	O.K.
13	Slight Pungent	Very Foul	Slight Pungent	Slight Mildew
14	Slight Pungent		O.K.	O.K.
16	Strong Pungent		Slight Pungent	Mildew (Musty)
19	Very Pungent		O.K.	Mildew (Musty)
24	Very Pungent		Pungent	Mildew (Musty)

*1 Slight Irradiation Odour

The results for the patty odour observed when the package was initially opened at various stages of storage are shown in Table 24. In general, both unirradiated control and 2.4 kGy irradiated patties maintained an acceptable odour longer when stored in dual bag packaging as compared to single bag packaging (Table 24). Unirradiated control patties stored in dual bag packaging developed a slight pungent odour by day 4 of storage, and after day 16 the pungent odour became dominant. The pungent odour in the dual bag packaged patties was consistent with lactic acid bacteria (LAB) dominating the spoilage microflora as found in section 5.4.2, and resulting in reduced patty pH (Fig 57).

The unirradiated control patties stored in single bag packaging developed an off odour by day 4 of storage. This was consistent with the APC of $1x10^8$/g by this storage time (Fig 5).

The irradiated patties did not develop offensive consumer odours until day 24 and day 16 in dual and single bag packaging, respectively. The dual bag packaged patties developed a pungent odour, while the single bag packaged patties developed a mildew or musty odour. The pungent odour in the dual bag packaged patties was consistent with LAB dominating the spoilage microflora (Fig 53) and resulting in reduced patty pH (Fig 57). The mildew or musty odour of the single bag packaged patties is consistent with the increased yeast and mould numbers observed (Fig 54).

5.5 Ascorbic Acid Experiment

The effect of incorporating an anti-oxidant (crystalline L-ascorbic acid) into ground beef was investigated as preliminary investigations showed poor red colour stability in irradiated and unirradiated control patties. Shivas et al. (1984) found colour improvement in refrigerated ground beef with the addition of ascorbic acid, and Elias (1986) stated that ascorbic acid reduced overall radiation effects by acting as a free radical scavenger. A level of 0.1% ascorbic acid (A.A.) was incorporated by regrinding the ground beef 5 times. Microbiological and colour of treated and control samples were evaluated.

5.5.1 Microbiological Analysis

Table 25. Ascorbic Acid Experiment, Fresh Ground Beef Irradiated (2.4 kGy) Dual Bag Packaged, Day 14 of Storage, 4°C

Reground 5 X	Yes	Yes	No
Ascorbic Acid	0.0%	0.10%	NA
Package	Dual	Dual	Dual
pH	5.86	5.70	5.74
	----------CFU/g----------		
APC	$1.2x10^5$	$1.2x10^5$	$1.2x10^5$
LAB-35 (Disc)	$2.8x10^4$	$4.8x10^4$	$7.6x10^3$
LAB-35 (Total)	$4.7x10^4$	$5.6x10^4$	$7.6x10^4$
Y&M	$2.2x10^6$	$1.8x10^6$	$1.6x10^6$
PSY	$3.4x10^6$	$4.2x10^6$	$2.2x10^6$

The control and 0.10% A.A. treated 2.4 kGy irradiated dual bag packaged patties involved in the ascorbic acid experiment were microbiologically evaluated on day 14 of storage, the results are shown in Table 25. The results for the irradiated dual bag packaged patties not involved in the ascorbic acid experiment, and thus not subjected to regrinding 5 times to incorporate the A.A. are also in Table 25. The data for the non-ascorbic acid experiment samples were previously shown in Fig. 48-57 for day 14. Shivas et Al. (1984) found the incorporation of up to 0.10% A.A. did not affect the APC of ground beef, while improving the colour retention. The similarity of the pH, APC, LAB-35 (Disc), LAB-35 (Total), Y&M, and PSY of all three treatments is shown in Table 25.

This not only confirms that there was no change in the microflora due to addition of 0.10% A.A. but also due to the regrinding of the ground beef in a sterile meat grinder.

5.5.2 Colour

The presence or absence of a bright red colour of patty is an attribute which effects the perception of quality ground beef.

5.4.2.1 Visual Colour Observation

The results of the visually observed colour of irradiated and unirradiated control dual bag packaged patties which had 0.10% or no ascorbic acid (A.A.) added and had the outer barrier bag removed on day 0 are shown in Table 26. All patties were dull brown at the time the outer barrier bag was removed on day 0. Both the irradiated and unirradiated control patties with no A.A. added remained dull brown until day 2 of storage and had progressive deterioration of the presence of redness in the colour, after that point, while stored in only the oxygen permeable inner bag. Slime appeared on the unirradiated control patties by day 8 of storage, while the irradiated patties never developed slime. A red surface mould did, however, appear on the irradiated patties by day 14 of storage.

Both the irradiated and unirradiated control patties containing 0.10% A.A. experienced an increase in red colour upon further storage in the oxygen

permeable inner bag after the outer barrier bag was removed on day 0 (Table 26). Within 1 day, patties with either dose treatment bloomed and were a bright red colour. The red tone persisted longer in the irradiated samples than the unirradiated control samples. The irradiated samples remained bright red for 8 days (day 1-day 8), while the unirradiated control patties remained bright red for 2 days (day 1-day 2) following the removal of the outer barrier bag and storage in the oxygen permeable inner bag. Brown tones dominated as the patty colour for both treatments later in storage. Slime appeared on day 10 while none was observed on the irradiated patties during the storage period monitored.

Table 26. Visual Colour Observations, Ascorbic Acid Experiment, Dual Bag Packaged Patties, Opened on Day 0 of Storage at 4°C

Dose	2.4 kGy	2.4 kGy	0 kGy	0 kGy
Ascorbic	0% AA	0.1% AA	0% AA	0.1%AA
Day				
0	Dull Brown	Dull Brown	Dull Brown	Dull Brown
1	Dull Brown	Bright Red	Dull Brown	Bright Red
2	Dull Brown	Bright Red	Dull Brown	Bright Red
6	Sandy Brown	Bright Red	Brown*1	Dull Brown
8	Sandy Brown	Bright Red	Brown*1(S)	Brown*1
10	Sandy Brown	Red	Red(S)	Red(S)
12	Sandy Brown	Sandy Brown	(S)	(S)
14	Sandy Brown*2	Sandy Brown*2		
16	Sandy Brown*2	Sandy Brown*2		
18	Sandy Brown(S)	Sandy Brown(S)		

*1 Red Tinge
*2 Red Surface Mould
(S) Slime

The results of the visually observed colour of irradiated and unirradiated control dual bag packaged patties which had 0.10% or no ascorbic acid (A.A.) added and had the outer barrier bag removed on day 6 are shown in Table 27. Both the irradiated and unirradiated patties containing 0% A.A. were predominantly brown when the outer barrier bag was removed on day 6. The brown colour remained dominant for both upon further storage in only the inner oxygen permeable inner bag. Slime appeared on the patties' surface on day 18 and day 14 for irradiated and unirradiated control samples, respectively.

Both the irradiated and unirradiated control patties containing 0.10% A.A. experienced an increase or bloom in red colour from a dull brown colour upon further storage in the oxygen permeable inner bag after the outer barrier bag was removed on day 6 (Table 27). Both the irradiated and unirradiated control patties bloomed within 24 hours and remained bright red for 4 days (day 7-day 10) following the removal of the outer barrier bag. As with the patties with no ascorbic acid added, the 0.10% A.A. patties developed slime on their surface by day 18 and day 14 of storage for irradiated and unirradiated control patties, respectively.

Table 27 Visual Colour Observations, Ascorbic Acid Experiment, Dual Bag Packaged Patties, Opened on Day 6 of Storage at 4°C

Dose	2.4 kGy	2.4 kGy	0 kGy	0 kGy
Ascorbic	0% AA	0.1% AA	0% AA	0.1%AA
Day				
6	Sandy Brown	Dull Brown	Brown*1	Dull Brown
7	Dull Brown	Bright Red	Dull Brown	Bright Red
8	Sandy Brown*1	Bright Red	Sandy Brown	Bright Red
10	Sandy Brown*1	Bright Red	Sandy Brown	Bright Red
12	Sandy Brown	Sandy Brown*1	Sandy Brown	Sandy Brown
14	Sandy Brown	Sandy Brown	Sandy Brown(S)	Sandy Brown(S)
16	Sandy Brown*2	Sandy Brown*2	Brown(S)	Brown(S)
18	Dull Brown(S)	Dull Brown(S)	Brown(S)	Brown(S)

*1 Red Tinge
*2 Red Surface Mould
(S) Slime

The results of the visually observed colour of irradiated and unirradiated control dual bag packaged patties which had 0.10% or no ascorbic acid (A.A.) added and had the outer barrier bag removed on day 12 are shown in Table 28. The patty colour was sandy brown and dull grey for irradiated and unirradiated patties containing 0% A.A., respectively, when the outer barrier bag was removed on day 12. Poor colour remained dominant for both upon further storage in only the inner oxygen permeable inner bag. Slime appeared on the patties' surface on day 18 for both irradiated and unirradiated control patties.

Both the irradiated and unirradiated control patties containing 0.10% A.A. experienced an increase or bloom in red colour from a sandy brown with slight red tinge colour upon further storage in the oxygen permeable inner bag after

the outer barrier bag was removed on day 6 (Table 28). Both the irradiated and unirradiated control patties bloomed within 24 hours and remained bright red for 2 days (day 13-day 14) following the removal of the outer barrier bag. As with the patties with no ascorbic acid added, the 0.10% A.A. patties developed slime on their surface by day 18 of storage for both irradiated and unirradiated control patties.

Table 28. Visual Colour Observations, Ascorbic Acid Experiment, Dual Bag Packaged Patties, Opened on Day 12 of Storage at 4°C

Dose	2.4 kGy	2.4 kGy	0 kGy	0 kGy
Ascorbic	0% AA	0.1% AA	0% AA	0.1%AA
Day				
12	Sandy Brown	Sandy Brown*1	Dull Grey*1	Sandy Brown*1
13	Sandy Brown*1	Bright Red	Dull Brown	Bright Red
14	Sandy Brown	Bright Red	Sandy Brown	Bright Red
16	Sandy Brown*2	Sandy Brown*2	Sandy Brown	Sandy Brown
18	(Slime)	(Slime)	(Slime)	(Slime)

*1 Red Tinge
*2 Red Surface Mould

The visually observed patty colours were dull brown and sandy brown at the time the outer barrier bag was removed on day 14 for irradiated dual bag packaged patties which contained 0.10% or no ascorbic acid (A.A.), respectively (Table 29). Further colour observations were not conducted after this point as the samples were used for microbiological evaluations.

Table 29. Visual Colour Observations, Ascorbic Acid Experiment, Dual Bag Packaged Patties, Opened on Day 14 of Storage at 4°C

Dose	2.4 kGy	2.4 kGy	0 kGy	0 kGy
Ascorbic	0% AA	0.1% AA	0% AA	0.1%AA
Day				
14	Sandy Brown	Dull Brown	NA	NA
(Samples Analyzed for Micro. After Day 14)				

NA Not Available

The visually observed patty colour was sandy brown at the time the outer barrier bag was removed on day 16 for irradiated dual bag packaged patties which contained 0.10% or no ascorbic acid (A.A.), respectively (Table 30). Both patties became dull brown with a red surface mould 2 days after (day 18).

Table 30. Visual Colour Observations, Ascorbic Acid Experiment, Dual Bag Packaged Patties, Opened on Day 16 of Storage at 4°C

Dose	2.4 kGy	2.4 kGy	0 kGy	0 kGy
Ascorbic	0% AA	0.1% AA	0% AA	0.1%AA
Day				
16	Sandy Brown	Sandy Brown	NA	NA
18	Dull Brown*2	Dull Brown*2	NA	NA

NA Not Available
*2 Red Surface Mould

The results of the visually observed colour of irradiated and unirradiated control single bag packaged patties which had 0.10% or no ascorbic acid (A.A.) added are shown in Table 31. All patties were dull brown on day 0. The

irradiated patties with no A.A. added remained dull brown until day 2 of storage and became sandy brown in their colour upon further storage in the oxygen permeable inner bag. Slime did not appear on the irradiated patties. A red surface mould did, however, appear on the irradiated patties by day 14 of storage. The unirradiated control patties with no A.A. added remained sandy brown until day 8 when slime appeared on the patties' surface.

Both the irradiated and unirradiated control patties containing 0.10% A.A. experienced a bloom or increase in red colour upon further storage in the oxygen permeable inner bag after the outer barrier bag was removed after irradiation on day 0 (Table 31). Within day 1 patties with either dose treatment bloomed and were a bright red colour. The red tone persisted longer in the irradiated samples than the unirradiated control samples. The irradiated samples remained bright red for 6 days (day 1-day 6), while the unirradiated control patties remained bright red for 1 day (day 1). A red colour immediately followed for both irradiated and unirradiated control patties, while brown tones dominated as the patty colour for both treatments later in storage. Slime appeared on day 10 for unirradiated patties while none was observed on the irradiated patties during the storage period monitored. The results are similar to the dual bag packaged patties which had the outer barrier bag removed on day 0 (Table 26).

Table 31. Visual Colour Observations, Ascorbic Acid Experiment, Single Bag Packaged Patties, Opened on Day 16 of Storage at 4°C

Dose	2.4 kGy	2.4 kGy	0 kGy	0 kGy
Ascorbic	0% AA	0.1% AA	0% AA	0.1%AA
Day				
0	Dull Brown	Dull Brown	Dull Brown	Dull Brown
1	Dull Brown	Bright Red	Dull Brown	Bright Red
2	Dull Brown	Bright Red	Dull Brown	Red
6	Sandy Brown	Bright Red	Dull Brown	Dull Brown*1
8	Sandy Brown	Red	Dull Brown(S)	Dull Brown*1
10	Sandy Brown	Red	Red(Slime)	Red(Slime)
12	Sandy Brown	Sandy Brown*1	(Slime)	(Slime)
14	Sandy Brown*2	Sandy Brown*2		
16	Sandy Brown*2	Sandy Brown*2		
18	Dull Brown*2	Dull Brown*2		

*1 Red Tinge
*2 Red Surface Mould
(S) Slime

5.5.2.2 Hunterlab Colour Difference Meter

The Hunterlab A value was monitored as it represents the degree of redness a sample possesses. The Hunterlab A values for dual bag packaged 2.4 kGy irradiated patties containing 0% Ascorbic acid (A.A.) and 0.10% A.A. are shown in figures 59 and 60. The outer barrier bags were removed on days 0, 6, 12, 14, and 16 of storage, exposing the oxygen permeable inner bag for the remainder of the storage period. The Hunterlab A value of the 2.4 kGy irradiated (0% A.A.) patties with the outer barrier bag removed on day 0 was 7.1. The Hunterlab A value increased slightly to 8 by days 1 and 2 followed by a steady decline to a low of about 4.4 on day 12. Further storage resulted in Hunterlab A increasing to 8 by

day 18. This agrees with the visually observed colour of dull brown on day 0 changing to sandy brown, followed by the appearance of red surface mould on day 14 (Table 26). The samples which had the outer barrier bag removed on day 6 had a Hunterlab A value of 7.5 at this time. The Hunterlab A increased to 8.7 on day 7 followed by a general decline to a low of 5.3 on day 16, with a final increase to 7.3 on day 18. These Hunterlab A values agree with the visually observed patty colour (Table 27). The increase in Hunterlab A between days 16 and 18 was due to the appearance of slime on the patty surface. The patties which had the outer barrier bag removed on day 12 had a Hunterlab A value of 6.8 at this time. There was an increase to 8.5 and 8.2 on day 13 and 14, respectively, followed by lower values on days 16 and 18. These Hunterlab A values agree with the visually observed results (Table 28). The Hunterlab A value was 6.3 for patties just after the outer barrier bag had been removed on day 14. This Hunterlab A value agrees with the sandy brown colour observed (Table 29). The patties which had the outer barrier bag removed on day 16 had a Hunterlab a value of 6.3 which increased to 7.1 by day 18. The increase was due to the appearance of red surface mould on the patties (Table 30). In general, 2.4 kGy irradiated dual bag packaged patties with no ascorbic acid added had poor (brown based) colour as indicated by low Hunterlab A values at the time the outer bag was removed (Fig 59). Slight increases in Hunterlab A values for the first day after removal of the outer barrier bag were followed by gradual declines in before Hunterlab A values would increase due to the appearance of slime or red surface mould.

Figure 59.

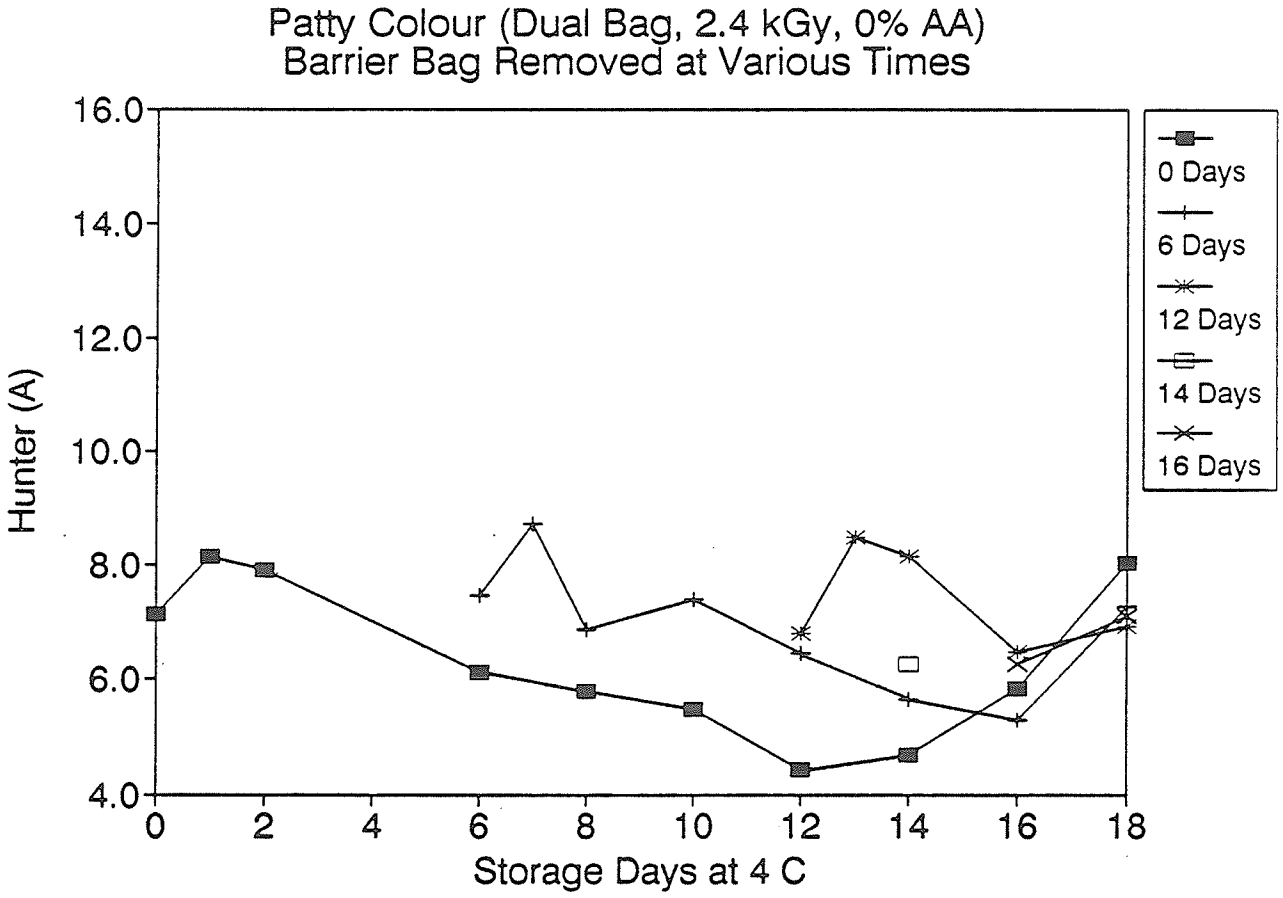
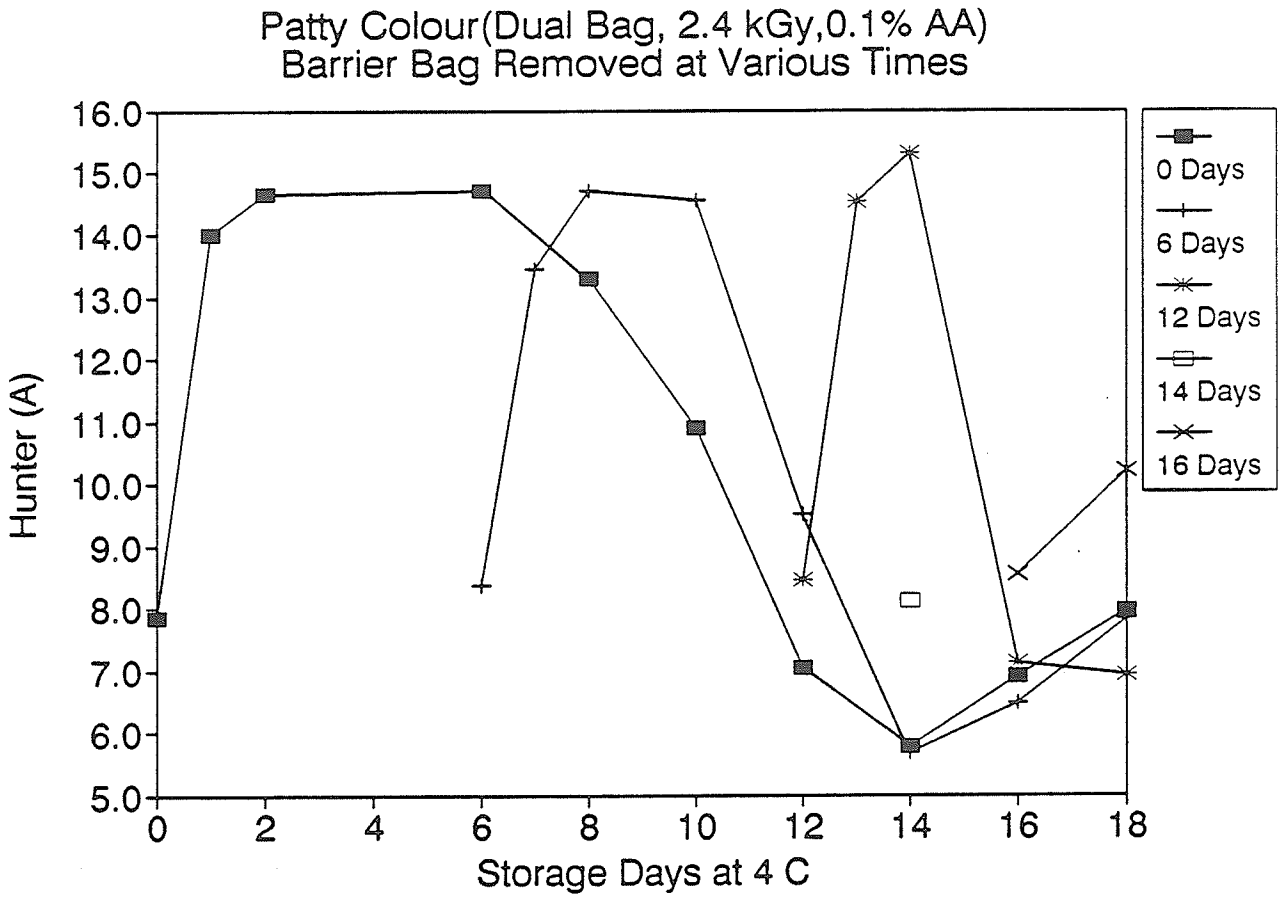

Patty Colour (Dual Bag, 2.4 kGy, 0% AA)
Barrier Bag Removed at Various Times
Hunter (A)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
0
2
4
6
8
10
12
14
16
18
Storage Days at 4 C
0 Days
6 Days
12 Days
14 Days
16 Days

Figure 60.


Patty Colour(Dual Bag, 2.4 kGy,0.1% AA)
Barrier Bag Removed at Various Times
Hunter (A)
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
0
2
4
6
8
10
12
14
16
18
Storage Days at 4 C
0 Days
6 Days
12 Days
14 Days
16 Days

The Hunterlab A value of the 2.4 kGy irradiated (0.10% A.A.) patties with the outer barrier bag removed on day 0 was approximately 7.8 (Fig 60). The Hunterlab A value increased to greater than 14 by day 1 and remained at this level until day 6 of storage. A steady decline followed to a low of about 5.8 on day 14. Further storage resulted in Hunterlab A increasing to 8 by day 18. This agrees with the visually observed colour of dull brown on day 0 improving to bright red between day 1 and 8 with a red patty colour on day 10 (Table 26). The patty colour deteriorated further to a sandy brown colour by day 12, with the appearance of red surface mould on day 14 (Table 26). The samples which had the outer barrier bag removed on day 6 had a Hunterlab A value of 8.4 at this time. The Hunterlab A increased to greater than 14 by day 8 and remained at this level until day 10 of storage. A rapid decline followed to a low of 5.7 by day 14. Further storage resulted in an increase in Hunterlab A increasing to 8 by day 18. These Hunterlab A values agree with the visually observed patty colour (Table 27). The patties which had the outer barrier bag removed on day 12 had a Hunterlab A value of 8.4 at this time. There was an increase to 14.5 and 15.3 on day 13 and 14, respectively, followed by a rapid decline to 7 on days 16 and 18. These Hunterlab A values agree with the visually observed results (Table 28). The Hunterlab A value was 8.1 for patties just after the outer barrier bag had been removed on day 14. This Hunterlab A value agrees with the dull brown colour observed (Table 29). The patties which had the outer barrier bag removed on day 16 had a Hunterlab A value of 8.6 which increased to 10.2 by day 18. The increase was due to the

appearance of red surface mould on the patties (Table 30). In general 2.4 kGy irradiated dual bag packaged patties with 0.10% ascorbic acid added had poor (brown based) colour as indicated by low Hunterlab A values at the time the outer bag was removed (Fig 60). Dramatic increases in Hunterlab A values indicate that bloom of a bright red colour occurred by the first day after removal of the outer barrier bag. The size of the plateau or period of bright red colour decreased the later the outer barrier bag was removed. The rate of colour decline following bloom is proportional to the date the outer bag is removed. That is, the rate at which Hunterlab A declines, or the decrease of redness following the bloom in colour, became more rapid the later in the storage period the outer bag was removed.

The Hunterlab A values for unirradiated control dual bag packaged patties containing 0% Ascorbic acid (A.A.) and 0.10% A.A. are shown in Figs. 61 and 62. The outer barrier bags were removed on days 0, 6, and 12 of storage exposing the oxygen permeable inner bag for the remainder of the storage period. The Hunterlab A value of the unirradiated control (0% A.A.) patties with the outer barrier bag removed on day 0 was 6.8 (Fig 61). The Hunterlab A value increased gradually to 9.1 by day 12. Monitoring of these patties ended at this time as they were spoiled. These Hunter A values agree with the dull brown patty colour visually observed (Table 26). The samples which had the outer barrier bag removed on day 6 had a Hunterlab A value of 8.5 at this time. The Hunterlab A increased to 10.1 on day 7 followed by a decline to a low of 4.8 on day 12, with a final increase to 9.0 on day 18. These Hunterlab A values agree with the visually

observed patty colour (Table 27). The increase in Hunterlab A between days 12 and 18 was due to the appearance of slime on the patty surface. The patties which had the outer barrier bag removed on day 12 had a Hunterlab A value of 8.3 at this time. There was an decrease to 5.6 by day 14, and remained at this level until day 18. These Hunterlab A values agree with the poor colour visually observed (Table 28). The unirradiated control dual bag packaged (0% A.A.) patties had low Hunterlab A values (poor red colour) at the time the outer barrier bag was removed and upon further storage in the oxygen permeable inner bag (Fig 61).

The Hunterlab A value of the unirradiated control (0.10% A.A.) patties with the outer barrier bag removed on day 0 was approximately 8.2 (Fig. 62). The Hunterlab A value increased to 13.7 by day 1 followed by a steady decline to a low of 7.6 on day 6. Further storage resulted in Hunterlab A value increasing to 9.0 by day 12, at which time monitoring these patties was terminated. This agrees with the visually observed colour of dull brown on day 0 improving to bright red on days 1 and 2. The patty colour deteriorated to dull brown on day 6, with surface slime appearing on day 10 (Table 26). The samples which had the outer barrier bag removed on day 6 had a Hunterlab A value of 7.3 at this time. The Hunterlab A increased to 14.0 by day 7 and steadily declined to a low of 5.7 on day 12. Further storage resulted in an increase in Hunterlab A to 8.2 by day 18. These Hunterlab A values agree with the visually observed patty colour (Table 27). The patties which had the outer barrier bag removed on day 12 had a Hunterlab A value of 8.6 at this time. There was an increase to 14.2 on day 13, followed by a rapid decline

to 6.5 on day 18. These Hunterlab A values agree with the visually observed results (Table 28).

The unirradiated control dual bag packaged patties with 0.10% ascorbic acid added had poor (brown based) colour as indicated by low Hunterlab A values at the time the outer bag was removed (Fig. 62). Dramatic increases in Hunterlab A value indicate colour bloom of a bright red colour occurred by the first day after removal of the outer barrier bag. There was no plateau or extended period of bright red colour after the outer barrier bag was removed, as found with the corresponding irradiated samples. There was only a steady decline in Hunterlab A value (red colour) after the initial bloom when the outer barrier bag was removed. This decreased stability of bloomed colour in unirradiated control samples compared to irradiated samples is consistent with increased microorganism load causing increased pigment oxidation. Lin et al. (1977) and Bala et al. (1977) found pseudomonads had a detrimental effect on colour in ground beef and beef steak, respectively.

(Single Bag Packaged)

The Hunterlab A value results for single bag packaged patties (2.4 kGy irradiated and unirradiated control, 0% and 0.10% A.A.) is shown in Fig. 63. The 2.4 kGy irradiated 0% A.A. single bag packaged patties as expected had similar Hunterlab A value results as those found for the corresponding dual bag packaged patties which had the outer barrier bag removed on day 0 (Fig. 59). The 2.4 kGy

irradiated 0% A.A. single bag packaged patties had a Hunterlab A value of 8.4 on day 0. There was a gradual decline after day 2 to a low of 4.8 on day 12. Further storage resulted in an increase in Hunterlab A value to 7.0 on day 18. This agrees with the dull brown colour visually observed between day 0 and 2 followed by a sandy brown colour until day 12 (Table 31). The appearance of red surface mould on day 14 explains the increasing Hunterlab A at later stages of storage. The 2.4 kGy irradiated 0.10% A.A. single bag packaged patties, as expected, had Hunterlab A value results similar to those of found for the corresponding dual bag packaged patties which had the outer barrier bag removed on day 0 (Fig 60).

The 2.4 kGy irradiated 0.10% A.A. single bag packaged patties, as expected, had similar Hunterlab A value results as those found for the corresponding dual bag packaged patties which had the outer barrier bag removed on day 0 (Fig. 60). The Hunterlab A value results of the 2.4 kGy irradiated (0.10% A.A.) single bag packaged patties was 10.1 on day 0 (Fig. 63). The Hunterlab A value increased to greater than 13 by day 1 and remained at a level of approximately 14 until day 6 of storage. A steady decline followed to a low of about 5.5 on day 14. Further storage resulted in Hunterlab A increasing to 7.8 by day 18. This agrees with the visually observed colour of dull brown on day 0 improving to bright red between days 1 and 6 with a red patty colour on days 8 and 10 (Table 31). The patty colour deteriorated further to a sandy brown colour by day 12, with the appearance of red surface mould on day 14 (Table 31).

Figure 61.

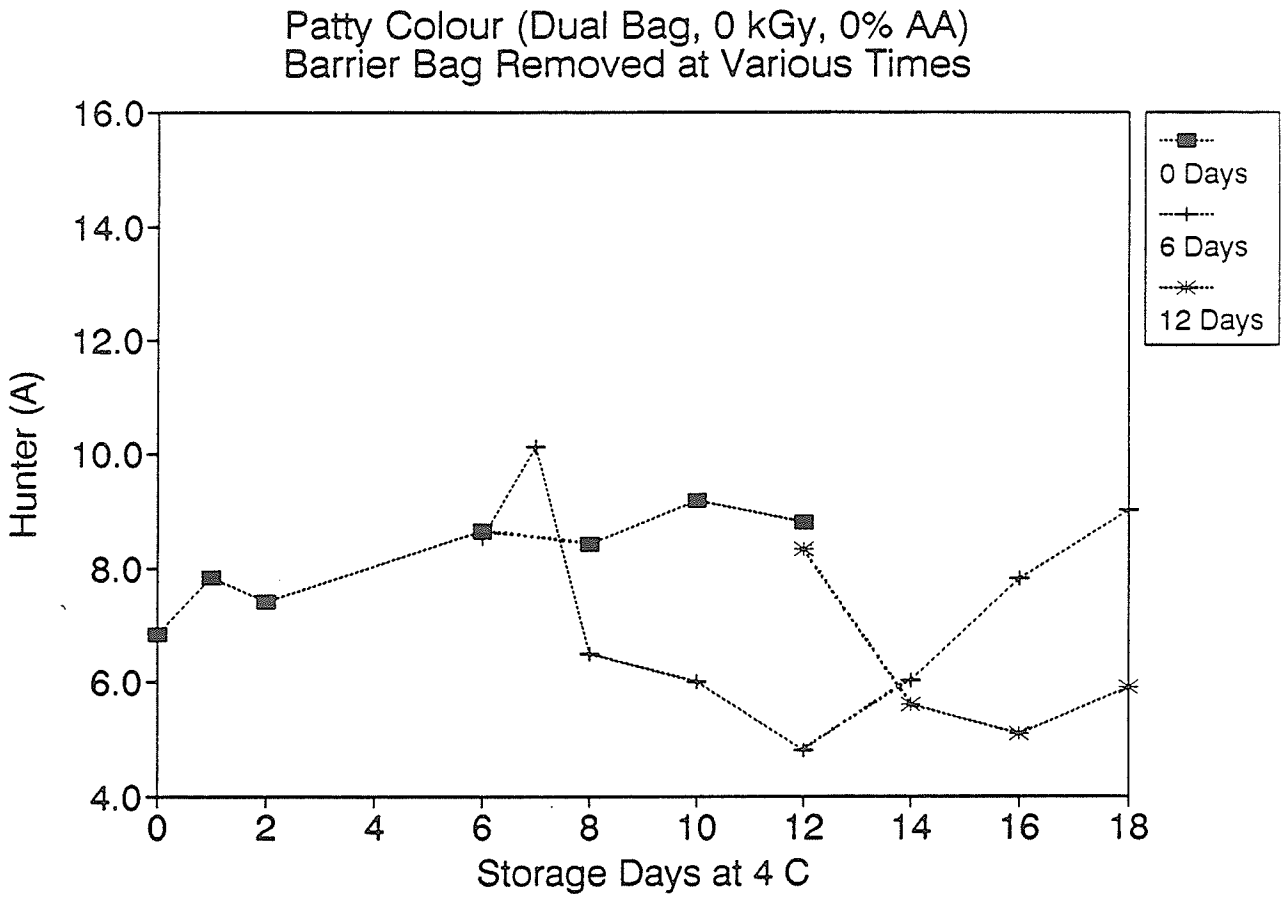

Patty Colour (Dual Bag, 0 kGy, 0% AA)
Barrier Bag Removed at Various Times
Hunter (A)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
0
2
4
6
8
10
12
14
16
18
Storage Days at 4 C
0 Days
6 Days
12 Days

Figure 62.

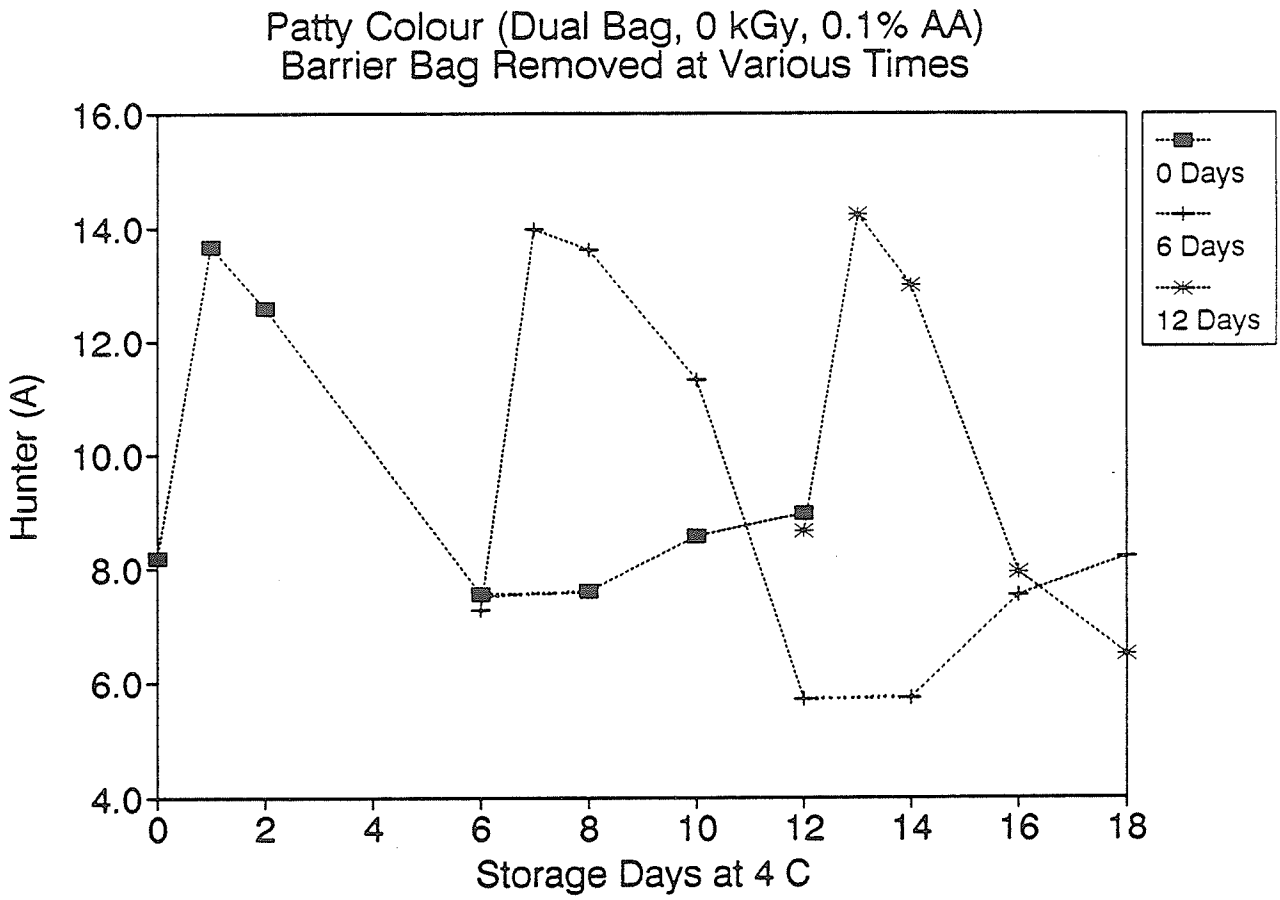
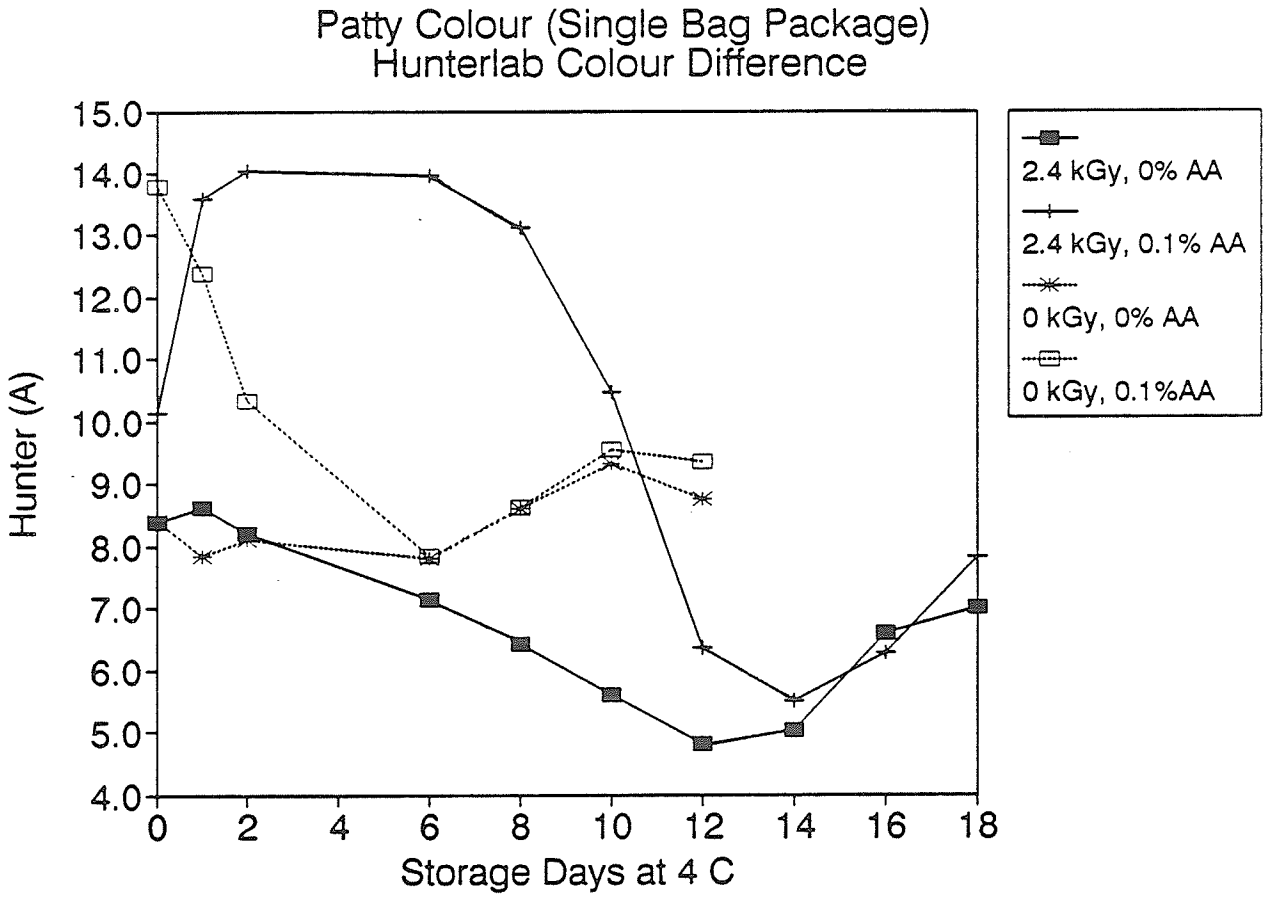

Patty Colour (Dual Bag, 0 kGy, 0.1% AA)
Barrier Bag Removed at Various Times
Hunter (A)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
0
2
4
6
8
10
12
14
16
18
Storage Days at 4 C
0 Days
6 Days
12 Days

Figure 63.


Patty Colour (Single Bag Package)
Hunterlab Colour Difference
Hunter (A)
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
0
2
4
6
8
10
12
14
16
18
Storage Days at 4 C
2.4 kGy, 0% AA
2.4 kGy, 0.1% AA
0 kGy, 0% AA
0 kGy, 0.1%AA

The unirradiated control (0% A.A.) single bag packaged patties, as anticipated, had similar Hunterlab A value results as those found for the corresponding dual bag packaged patties which had the outer barrier bag removed on day 0 (Fig. 61). The Hunterlab A value of the unirradiated control (0% A.A.) single bag packaged patties was 8.4 (Fig. 63). The Hunterlab A value increased gradually to 8.7 by day 12. Monitoring these patties ended at this time as they were spoiled. These Hunter A values agree with the dull brown patty colour visually observed (Table 31).

The unirradiated control (0.10% A.A.) single bag packaged patties, as expected, had similar Hunterlab A value results as those found for the corresponding dual bag packaged patties which had the outer barrier bag removed on day 0 (Fig. 62). The Hunterlab A value of the unirradiated control (0.10% A.A.) single bag packaged patties was 13.8 on day 0 (Fig. 63). The Hunterlab A value steadily declined to a low of 7.8 on day 6. Further storage resulted in Hunterlab A increasing to 9.4 by day 12, when monitoring of these patties was terminated. This agrees with the visually observed colour of dull brown on day 0 improving to bright red and red on day 1 and 2, respectively. The patty colour deteriorated to dull brown on day 6, with surface slime appearing on day 10 (Table 31).

(Summary)

The 2.4 kGy irradiated single bag packaged patties with 0% A.A. had low Hunterlab A values and poor red patty colour over the 18-day monitored storage

period. The addition of 0.10% A.A. resulted in high Hunterlab A values (red colour) for 10 days of storage. The unirradiated control single bag packaged patties with 0% had poor colour (low Hunterlab A values) during the entire storage period, while the addition of 0.10% A.A., extended the acceptable colour until only day 2 of storage. This decrease in colour stability in unirradiated samples is consistent with increased pigment oxidation caused by the higher bacterial microflora (Bala et al., 1977; Lin et al., 1977).

Both 2.4 kGy irradiated and unirradiated control patties with 0.1% A.A. had similar acceptable colour shelf life. The colour was acceptable until days 10 and 14 of storage, subsequent to the outer barrier bag being removed on days 6 and 12 of storage, respectively. Slightly higher Hunterlab A values and redder patty colour were observed with the irradiated samples as compared to the unirradiated controls. Both 2.4 kGy irradiated and unirradiated dual bag packaged patties with 0% A.A. had low Hunterlab A values and poor observed patty colour for the entire storage period, even after the outer barrier bag was removed on day 6 or 12 of storage.

6. DISCUSSION

The objective was to investigate the shelf life extension of refrigerated fresh ground beef patties by radurization. To achieve this, patties were irradiated in an anaerobic packaged state to limit the formation of radiolytic products which lead to lipid peroxidation. There was concern of possible C. botulinum growth and toxin production if the package remained anaerobic and temperature abuse occurred. Oxygen was reintroduced into the packages after irradiation in an attempt to inhibit this growth. The reintroduction of oxygen was facilitated by packaging the patties in a dual bag packaging arrangement. The inner bag was gas permeable with an outer gas barrier bag. Non-pathogenic C. sporogenes was used as an indicator organism for C. botulinum in all inoculation pack studies for safety reasons. Prior to determining the refrigerated shelf life extension, safety concerns regarding temperature abuse were addressed.

6.1 Safety

6.1.1 Inoculation Pack Studies (Sterilized Ground Beef)

The inoculation pack studies were conducted using radiation sterilized ground beef patties, which contained no competitive microflora. Dual bag

packaged patties were stored at 2°, 10°, 15°, and 20°C. No growth of inoculated C. sporogenes was observed at 15°C or lower for storage up to 4 weeks. At 20°C storage the total (vegetative cells and spores) C.sporogenes increased between days 3 and 6 of storage. There was a delay before the number of C. sporogenes spores increased. They did not increase until between days 6 and 8. This experiment was repeated using patties stored in single bag packages as well as dual bag packages. This would allow the effect of the reintroduction of air through the gas permeable inner bag on clostridial growth to be determined. Again there was no growth of inoculated C. sporogenes at a storage temperature of 15°C or less over a 4-week storage period. Total C. sporogenes in inoculated patties stored at 22°C in either packaging condition increased again between days 3 and 6 of storage. This indicated that the reintroduction of oxygen into the package did not inhibit the growth of C.sporogenes. This was contrary to what was expected, as Sarathchandra et al. (1974, 1977), found oxygen inhibited germination and growth of C. sporogenes in media. The lack of growth at storage temperatures less than or equal to 15°C was consistent with findings of other researchers. Baker et al. (1986) found inoculated C. sporogenes numbers decreased in media and ground chicken when stored for 6 days in air or 80% carbon dioxide (balance air) at 2°, 7°, and 13°C. Silliker and Wolfe (1980) found no growth of inoculated C. sporogenes in MAP (various O_2, CO_2, and N_2 compositions) packaged ground beef stored at 10°C for up to 10 days. Molins et al. (1986) and DeFreitas et al. (1988b) both found no growth of inoculated

C. sporogenes after 7 days of vacuum packaged storage at 5°C of bratwurst and liver sausage respectively. Whiting et al. (1984) also found no growth of inoculated C.sporogenes in vacuum packaged frankfurters up to 11 days of storage at 5°, 11°, and 16°C. The inhibitory effect of additives in the frankfurters was suspected since in a later investigation, Whiting et al. (1985) did find slight growth at 11°C (14 days) and moderate growth at 16°C (8 days) storage in inoculated vacuum packaged cooked corned beef.

6.1.2 Inoculation Pack Studies (Radurized Ground Beef)

In two inoculation pack studies conducted with radurized ground beef patties, the natural microflora of radurized ground beef would be present as competition for the inoculum. Again no C. sporogenes growth was found in patties stored at 15°C or lower for up to 4 weeks in dual or single bag packaging. At 22°C storage however, growth of inoculated C. sporogenes was observed in both dual and single bag packaging conditions.

In Test #1 at 22°C storage, the increase in total C. sporogenes occurred between days 8 and 12 and between days 6 and 8, for dual and single bag packaging, respectively. Detectable microbiological spoilage (APC $>5x10^7$/g) was reached prior to day 3 of storage for both dual and single bag packaging. This meant there was a "safety period" of detectable spoilage prior to C. sporogenes growth (indicator of botulinum risk). This safety period was at least 5 and 3 days, respectively for dual and single bag packaged radurized patties.

In Test #2 the increase in total C. sporogenes occurred between days 3 and 6 of storage at 22°C in both dual and single bag packaging. Again, detectable microbiological spoilage occurred prior to day 3 of storage in both packaging conditions. The safety period, in this case, was between 1 and 4 days for radurized patties for either packaging condition. The growth of clostridia at an earlier stage of storage in Test #2 may be due to higher patty pH in this test as opposed to Test #1. Enfors and Molins (1978) and Madril and Sofos (1986), found reducing pH reduced the germination and growth of C. sporogenes in media and food, respectively.

The lack of reintroduction of oxygen (dual bag) does not reduce the safety of radurized ground beef patties compared to reintroduction of oxygen (single bag). In fact, results from Test #1 suggest dual bag packaged patties had a larger safety period of detectable spoilage prior to clostridial growth. This may be due to the ability of lactic acid bacteria (LAB) to dominate in radurized meat stored in these low oxygen conditions. The more radiation-resistant LAB can survive the radurization dose and thrive in the low oxygen packaging. The Ph reduction of the patties by LAB, and perhaps production of inhibitory compounds by these organisms, may be the reason why clostridia growth was delayed in both types of packaging in radurized patties compared to sterilized patties with no competitive microflora. This pH reduction was cited by Al-Mashat (1972) as the mechanism utilized by an added Pediococcus culture to delay toxin production by C. botulinum type A spores in pasteurized ground beef.

The storage time required at room temperature abuse before C.sporogenes growth was found to be in the same vicinity as that observed by other researchers studying various food products (DeFreitas et al., 1988; Molins et al., 1986; Silliker and Wolfe, 1980; Sofos, 1986; Vareltzis et al., 1984).

There was a time delay of several days between the increase in total (vegetative cells and spores) C. sporogenes and increase in C. sporogenes spores in all inoculation pack experiments stored at abuse temperatures (sterilized and radurized). This delay demonstrates the need to monitor the total clostridia, not just the spores, to obtain the most pertinent information regarding safety of the product. There was a difference noted between results in this thesis and those of DeFreitas et al. (1988a,b) and Molins et al. (1986) with regard to the number of spores present when the vegetative clostridia increase. These researchers found the number of spores decreased as vegetative cells concurrently increased. This was consistent with all the inoculated spores germinating, followed by outgrowth and multiplication of the germinated C. sporogenes. Molins et al. (1987) found the number of inoculated clostridial spores decreased to 0 without any concurrent increase in vegetative clostridia, suggesting germination of spores but no outgrowth. In all of the experiments for this thesis, the number of spores remained essentially unchanged as the number of total clostridia increased, followed by an increase in the number of spores after the above mentioned time delay. These results were consistent with a few inoculated C. sporogenes spores germinating, and outgrowing, followed by the multiplication of these vegetative

cells. After environmental conditions became unfavourable, these vegetative cells sporulated, resulting in the delayed increase in number of spores.

The safety of dual and single bag packaged radurized (2.5 kGy) ground beef patties with respect to C. botulinum has been demonstrated (using C. sporogenes as an indicator organism for C. botulinum). The safety of radurized ground beef with respect to vegetative pathogens (Salmonella, Campylobacter, Staphylococcus, etc.) has been reviewed (Farkas, 1989; Maxcy 1983).

6.2 Shelf Life Extension

The shelf life of dual and single bag packaged ground beef patties stored at 4°C was investigated. The microbiological quality, colour, and odour of patties were monitored. The shelf life, microbiological aspects, and odour (including descriptive of spoilage odour) are shown in Table 32. The microbiological shelf life (APC $<5x10^7$/g) in dual bag packaged patties was greater than the corresponding irradiated or unirradiated control, single bag packaged patties. Irradiation extended the microbiological shelf life of single bag package patties to 16 days from <3, and irradiated dual bag packaged patties possessed a lower APC on day 24, compared to unirradiated dual bag packaged patties. Both the dual and single bag packaged radurized patties offer the added safety advantage due to the elimination of vegetative pathogens. The effective shelf life of the dual bag packaged patties, however, was limited to 14 and 19 days, respectively,

by the days of acceptable odour. The patty pH for both irradiated and control dual bag packaged patties remained below the initial value of 5.9 during the storage period monitored. The patty pH for the single bag packaged patties increased to greater than the initial value by day 10 and 2 for irradiated and control samples, respectively. The odours noted and patty pH were consistent with those expected, due to the dominant spoilage microflora in each case. Lactic acid bacteria were expected to dominate irradiated and control dual bag packaged patties, while yeast and pseudomonads were expected to dominate the irradiated and control single bag packaged patties.

The microbiological shelf life extension obtained is consistent with that found by other researchers. Niemand et al. (1983) found shelf life extension to slightly more than 11 days from 2 days and to >21 days from 7 days for ground beef irradiated at 2.5 kGy and stored at 4°C in aerobic and vacuum packaged conditions, respectively. For 3 kGy treatments, Okafor (1973) found shelf life extension from approximately 2 days to 15 days in vacuum packaged ground beef, while Niemand et al. (1980) found extensions to >28 days in vacuum packaged ground beef stored at 4°C. Due to the variability in microflora composition and contamination levels in ground beef, the effect of a given irradiation dose on a population and effect on subsequent shelf life can not be absolutely predicted.

Table 32. Shelf Life of Ground Beef Patties, Stored at 4°C

Dose (kGy)	Type of Package	Shelf Life (Days) Microbiological	Odour	Odour Descriptive
0	Dual	>24*	14	Pungent
0	Single	<3	~3	Rotten
2.4	Dual	>24**	19	Pungent
2.4	Single	16	16	Musty

* Day 24: APC, $1x10^7$/g
** Day 24: APC, $9x10^5$/g

The "colour shelf life" or days of acceptable colour for patties of various treatments are shown in Table 33. Poor, dull brown colour was observed in all patties with no ascorbic acid added. The single bag packaged patties had their acceptable colour shelf life extended to 10 and 2 days, in irradiated and unirradiated control samples, respectively. Both the irradiated and control dual bag packaged patties had days of acceptable colour extended to 10 and 14 days, for storage in dual bag packaging with removal of the outer barrier bag on days 6 and 12, respectively.

Richards and Morrison (1971) studied the effect storage time at 4°C on irradiated dual bag packaged (inner film permeable, outer film gas barrier) ground beef. At 5 kGy they found that the dual bag packaged patties remained redder (higher Hunter A value) than the single bag packaged patties. They also found that removal of the outer barrier bag on day 8 allowed for the re-

oxygenation (bloom) of ground beef colour. These findings were similar with the results found in this thesis research.

Shivas et al. (1984) found the addition of 0.05% and 0.10% crystalline ascorbic acid improved sensory scores for red colour and CIE a* values compared to control samples. The ground beef was packaged in gas permeable film and stored over a period of 10 days at 3°C. This positive effect of ascorbic acid on colour stability was also shown in the results for irradiated and unirradiated control patties stored in dual and single bag packaging.

Table 33. Colour Stability, Effect of Ascorbic Acid (A.A.) on Patty Colour Retention

Dose (kGy)	Type of Package	Shelf Life: Days of Acceptable Colour		
		0 % A.A.	0.10 % A.A.	
0	Dual	<1	10*	14**
0	Single	<1	2	
2.4	Dual	<1	10*	14**
2.4	Single	<1	10	

* Outer barrier bag removed on day 6
** Outer barrier bag removed on day 12

6.3 Method Development to Monitor C. sporogenes in Ground Beef

The growth of the vegetative cells is associated with the production of toxin by C. botulinum (Duda and Slack, 1969; Siegel and Metzger, 1979). To effectively monitor the safety using C. sporogenes inoculation pack studies,

vegetative clostridia must be monitored. The high background natural microflora in fresh and radurized ground beef over storage interfered with the enumeration of the rhizoid shaped C. sporogenes colonies on the non heat-treated Pflug medium assay. A selective/differential medium was developed to monitor inoculated C. sporogenes in a food with a high background microflora such as ground beef. The assay medium developed SC+2, utilized SC medium which is the Health Protection Branch MFA-23 C. perfringens medium, and included supplements identified by Pflug et al. (1979) that improve recovery of C. sporogenes. The C. sporogenes produces black colonies on this medium (differential aspect). The anaerobic incubation, and presence of d-cycloserine, and sodium metabisulfite provide the selective aspect.

SC+2 medium proved effective in monitoring total (vegetative cells and spores) C sporogenes, (non heat-treated assay) and C. sporogenes spores (heat-treated assay) in stored radurized ground beef. However, there were some small black interfering colonies present in the total C. sporogenes assay in unirradiated ground beef. Hydrogen sulfide producing pseudomonads are the/(one of the) suspected organisms that produced these interfering black colonies. Proper isolation and identification of the interfering organism/s should be conducted, if further modification to the medium is desired to allow this medium to be effective in unirradiated ground beef as well as radurized ground beef. Possible further modifications include:

a.) Addition of Kanamycin and/or Polymixin B to increase the selectivity of SC+2 medium, as Shahidi and Ferguson (1971) recommended their use in conjunction with SFP medium (SC medium base).

b.) Addition of egg-yolk supplement used in the TSC agar cited for enumeration of C. perfingenes in food by FDA and BAM (Harmon, 1984; Harmon and Duncan, 1984). They noted that C. sporogenes will also give an egg yolk positive reaction (2-4 mm opaque white zone surrounding the black colonies due to lecithinase activity). This added distinctive aspect may be sufficient to differentiate C. sporogenes from the interfering background organism/s.

7. CONCLUSION

Refrigerated shelf life of lean ground beef patties stored at 4°C can be extended by radurization (2.5 kGy). Radurization and storage in dual bag packaging (anaerobic) is more effective in extending shelf life than removing the outer gas barrier bag subsequent to radurization with storage in gas permeable single bag packaging (aerobic). Patties stored in dual bag packaging following radurization had a microbiological shelf life of greater than 24 days but the effective shelf life was limited to 19 days due to odour unacceptability. In the single bag packaged case, microbiological shelf life was 16 days but again the effective shelf life was limited by unacceptable odour, which was present after 14 days.

The patty colour in either dual or single bag packaging was poor. Lack of red colour was noted during the entire storage period. The stability of patty colour was improved by the addition of 0.10% crystalline ascorbic acid. The addition of ascorbic acid extended the acceptable colour of radurized dual bag packaged patties to 10 and 14 days when the outer barrier bag was removed on days 6 and 12 of storage, respectively. The patties stored in single bag packaging had acceptable colour for 10 days. The safety concern for the potential of botulism, if radurized patties packaged in anaerobic conditions were temperature

abused, was addressed with inoculation pack studies using C. sporogenes spores as an indicator organism for C.botulinum. No growth of C. sporogenes was observed in radurized patties stored for 4 weeks up to 15°C. The safety of radurized patties stored at 22°C stored in both dual (anaerobic) and single bag (aerobic) packaging conditions was demonstrated. Detectable spoilage occurred prior to growth of C. sporogenes.

The inhibitory effect of the competitive microflora present in dual and single bag packaged radurized patties on growth of inoculated C. sporogenes was demonstrated. The microflora of patties stored in dual bag packaging appear to delay the growth of C. sporogenes to a greater extent than the microflora in single bag stored samples, which had air reintroduced.

A selective and differential media was developed to monitor the inoculated C. sporogenes. The media may be used to monitor number of total (vegetative cells and spores) clostridia or clostridial spores only, in a food product with a high background of microflora, such as stored radurized ground beef.

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