The Impact of Modified Atmosphere Packaging and Irradiation on the Survival and Pathogenicity of *Listeria monocytogenes* and the Design of a Mathematical Model

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THE IMPACT OF MODIFIED ATMOSPHERE PACKAGING AND IRRADIATION ON THE SURVIVAL AND PATHOGENECITY OF *Listeria monocytogenes* AND THE DESIGN OF A MATHEMATICAL MODEL

BY

MEI CHUNG-LEWIS

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of MASTER OF SCIENCE

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The survival of *Listeria monocytogenes* in packaged, ground containing either CO_2 (15% or 30%) or air was examined during storage at 5 or 11°C. Survival was evaluated when electron beam radiation (1.75 kGy) was used as part of a combination treatment. Compared to ground beef stored in air, particularly at 5°C, listeriae survival in CO_2 was approximately 1 to 2 logs lower after 21 d. Similarly, the growth ratio of the organism increased as the CO_2 level decreased. In contrast, increasing the fat content of the samples from 13% (54% protein) to 30% (20% protein) appeared to result in a decrease in effectiveness of CO_2. Neither 15 nor 30% CO_2 was effective in retarding listeriae growth when beef samples were stored at 11°C. Irradiation of the meat samples decreased the listeriae population by ca. 2 logs regardless of the composition of the packaging atmosphere. In the presence of CO_2, however, the survivor growth was much reduced compared to that in air. Increasing the protein content of the meat samples did not appear to affect survival levels following irradiation treatment regardless of the storage temperature or CO_2 concentration. The inhibitory effect of CO_2 was observed to decrease as the storage temperature was increased to 11°C. This effect was similiarly observed with the irradiated samples. Hemolysis, a known virulence factor for listeriae, was exhibited by survivors following storage in CO_2 with or without irradiation. Quantitative pathogenic analyses, using chick embryos inoculated with listeriae isolated from the

various treatment protocols, indicated that increasing the storage temperature of the meat samples from 5 to 11^{0}C resulted in increased deaths.

The mathematical model, $\frac{S}{S_o} = \exp(\mu_o^* (1 - \exp(-Dt))$, is derived from the experimental data generated by this study. Fat (protein) content, storage temperature, and atmospheric conditions were the parameters which were applied to derive this equation. The growth ratio can be used to predict the effectiveness of these parameters on the survival of the organism. For example, this equation demonstrates that the number of listeriae increased as the level of CO_2 decreased in both 5 and 11^{0}C. By manipulating the proportionality constant, μ_o^*, and using the exponential data points, a best fit line can be produced to the one that is very similar to the growth curve of an organism. The goodness of fit of this line can demonstrate the effect of various treatment protocols to the survival of the organism.

INTRODUCTION

Contamination of foods, especially those of animal origin, with bacterial pathogens is important because of potential public health problems (El-Shenawy et al., 1989). In this regard, foodborne diseases are an area of great concern among government agencies and in particular by the general public (Sockett, 1995). This concern has been generated by three main factors. The first factor is the increase in reported sporadic incidences and outbreaks of foodborne illness. The World Health Organization Surveillance Program, for example, has reported increasing number of incidents for the years 1985 to 1989 (WHO, 1992). The second factor is the observed trends in disease aetiology. Dominance of salmonellae infection accounted for 75% of over 7000 outbreaks reported by sixteen European countries between 1985 and 1989, where the aetiology of infection was known (Sockett, 1995). Also, newly recognized foodborne pathogens have emerged as important causes of illness. These include *Listeria*, *Yersinia* and verocytotoxin-producing *Escherichia coli*. Third, the recognition that the costs of foodborne disease are significant (Sockett, 1995). Since contamination of food can occur in any of the many stages between the time raw material is acquired and the time food is consumed, maintaining safety and quality poses new challenges to food microbiologists. In particular, new processes which have the potential to extend food quality are now being examined more closely.

For example, considerable interest in modified atmosphere packaging (MAP) of muscle foods is now being generated in Canada and United States. MAP with a carbon dioxide enriched atmosphere provides an alternative method to the packaging

of meat products and provides extension of shelf-life (Genigeorgis, 1985). In this technique, artifical atmospheres are flushed into a gas impermeable package containing food which is then sealed; or the artificial atmosphere may be injected into a shipping container. Several gases including carbon dioxide, oxygen, nitrogen and carbon monoxide can be used in the preparation of such artificial atmospheres.

When compared to air, elevated levels of CO_2 (greater than 10%) restrict the growth of common spoilage bacteria, such as gram-negative rods like *Pseudomonas* and mold (Baker et al., 1985). Studies have demonstrated that elevated CO_2 levels can reduce the number of aerobic colony forming units by a factor of 10^2 to 10^3 / ml or g. This reduction in microbial load is often accompanied by a change in the microflora. For example, gram-positive organisms usually predominate at elevated levels of CO_2, but in foods without elevated CO_2, gram-negative organisms tend to be predominant especially if the temperature of storage is $< 10^0$C (Baker et al., 1985).

The principal disadvantage of using elevated levels of CO_2 in the packaging of fresh meat is the development of undesirable colours due to metmyoglobin formation on the tissue surface and possible oxidation of lipids (Seideman and Durland, 1984). Furthermore, studies have found that the efficacy of CO_2 is temperature dependent. Clark and Lentz (1969) reported that the use of 20% CO_2 contributed to longer product shelf-life at lower temperatures (0^0C); however, there was no detectable benefit at 20^0C. This was due primarily to the effect of temperature itself on the microbial growth rate. In view of these disadvantages, alternative methods including irradiation have been investigated for use either alone or in concert with other

preservation techniques especially in situations where temperature abuse could occur (El-Shenawy et al., 1989). The purpose of treating foods with radiation would be to ensure microbiological safety and also to extend product quality (Urbain, 1983).

Studies have shown that low dose radiation (2 – 3 kGy) will reduce initial microbial populations (Thayer, 1995; Thayer et al., 1995; Lee, 1995). A longer than usual time period therefore is needed for the development of microbial spoilage. This delay constitutes product life extension. In addition, these low doses would be sufficient to decontaminate meat with pathogens such as salmonellae, *Campylobacter* and *E. coli* (Ingram and Farkas, 1977; Loaharanu, 1995; Monk et al., 1995). Radiation would also be applicable to packaged or unpackaged chilled, frozen or dried foods causing very little visible change, with only minimal, or no, sensorial effects especially in low lipid containing foods (Lagunas-Solar, 1995). However, the application of this technology has been limited, even though it has the advantage of being able to penetrate large pieces of meat or whole poultry. Its limited use appears to be due to distrust by the public of any process which depends on the nuclear industry as well as the lack of knowledge in general concerning foodborne infections and the effectiveness of irradiation (Bruhn, 1995; Resurreccion et al., 1995).

Combination treatments have been proposed as a means of enhancing the preservative effect of irradiation (Niemand et al., 1983 and Thayer et al., 1991). One example would be the use of MAP in conjunction with low dose irradiation to reduce the numbers of spoilage and pathogenic microorganisms (Grant and Patterson, 1991; Patterson, 1988). Combined treatments involving irradiation and MAP have been used

in various investigations including those with pork (Grant and Patterson, 1991). In such studies, researchers found that a modified atmosphere containing a minimum of 25% CO_2 controlled microbial growth in irradiated pork stored at 4^0C, compared to unirradiated MAP samples. Zhao et al. (1996) found that using a combination of irradiation (1.0 kGy) with vacuum or elevated CO_2 packaging (25%, 50% and 75%) resulted in no survival of salmonellae on pork loin chops after 2 weeks of storage at 2 to 4^0C. In addition, no recovery was observed after one more day of incubation at an abusive temperature of 25^0C. Decreasing the irradiation dose in combination with MAP may therefore produce fewer deleterious effects overall on the sensory and micronutrient properties of the product yet still achieve control of foodborne pathogens including *Listeria monocytogenes* (Zhao et al., 1996).

L. monocytogenes is a gram-positive microorganism that is widely distributed in nature. It is pathogenic for humans and animals (Chakraborty and Goebel, 1988). In humans, foodborne illness is the most common form of listeriosis which may result in gastroenteritis. *L. monocytogenes* can also cause bacterial meningitis and prenatal infections which may result in abortion, stillbirth, and infant death. Studies have been carried out on the effects of MAP on the growth of *L. monocytogenes*. For example, Marshall et al. (1991) found that although the growth of *L. monocytogenes* was moderately inhibited by MAP (76%:13.3%:10.7% CO_2:N_2:O_2 and 80%:20% CO_2:N_2) as compared to air, the organism was still capable of growth at 3, 7, and 11^0C. Kallander et al. (1991) also found that an increased level of CO_2 (70%) was ineffective in controlling the growth of *L. monocytogenes* at 5^0C when inoculated in shredded

cabbage. With regards to its radiation sensitivity, El-Shenawy et al. (1989) reported that this organism was more sensitive in broth (D-value of 0.34-0.5 kGy) than in ground beef (D-value of 0.51-1.0 kGy). Tarjan (1990) found that some strains of *L. monocytogenes* are less sensitive to irradiation and can survive doses as high as 4 kGy.

Since all strains of *L. monocytogenes* are viewed as pathogens, its hemolytic properties have attracted considerable attention. In part this is because of its association with virulence (Chakraborty and Goebel, 1988). All nonhemolytic strains are considered nonpathogenic while all pathogenic strains are capable of producing varying amounts of hemolytic activity. The hemolytic activity is attributed to a single molecule called listeriolysin and studies have demonstrated that hemolytic activity was highest when bacteria were grown to stationary phase (Geoffroy et al., 1989; Leimeister-Wachter and Chakraborty, 1989). Interestingly, the loss of the hemolysin activity was followed by a total absence of pathogenicity (McMeekin et al., 1993). Apparently, this loss can occur spontaneously or it can be induced by irradiation (Hunter at al., 1950) or by genetic engineering (Berche et al., 1988; Goebel et al., 1988). Leimeister-Wachter et al. (1992) also found that the hemolytic activity of *L. monocytogenes* strains varied with growth temperature. In cultures that had been grown overnight at either 20 or 30^{0}C, little or no hemolytic activity was detected. However, when grown at 37^{0}C, the activity increased 8 to 16-fold, depending upon the strain.

The ability to derive equations which are capable of predicting microbial growth over a range of temperatures may give a better understanding of the

risks involved when subjecting microorganisms to various environmental conditions (Broughall et al., 1983). Predictive microbiology is an alternative to developing technology as it may provide faster microbiological analyses and yet prove to be less costly (McMeekin et al., 1993). Overall, predictive microbiology aims to summarize the probable behaviour of specific spoilage organisms and the progression of spoilage processes in foods (McMeekin and Ross, 1996). It relies upon the development of mathematical models which can predict the rate of growth or the decline of microorganisms under a given set of environmental conditions. Models are derived by measuring the responses of microorganisms to various conditions like temperature, pH, gaseous atmosphere, chemical preservatives and water activity. These factors may act singly or in combination to affect the growth rate of the organism. McMeekin et al. (1992) reviewed the application of predictive microbiology in assuring the quality and safety of fish and fish products. Chandler and McMeekin (1989), Fu et al. (1991) and Griffiths et al., (1987) also applied equations to predict the shelf-life of dairy products. Models for red meat were considered by Gill (1986). Mathematical modeling has also been used to predict the probability of pathogen growth including *Staphylococcus aureus* (Genigeorgis et al., 1971) and *Clostridium botulinum* in pasteurized, cured meats (Roberts at al., 1981). Since temperature and storage conditions may vary extensively throughout the complete production and distribution chain, it follows that a general modeling approach is required.

The specific objectives of this investigation were to:

1. Assess the effectiveness of a combination treatment protocol involving MAP and irradiation for packaged ground beef to control growth of *Listeria monocytogenes*. In this respect, studies were carried out on ground beef maintained at either 5^0C or 11^0C. The latter temperature was used to reflect abusive refrigeration conditions.

2. To determine whether the treatments used to prolong the shelf-life of the product including the storage conditions had an impact on the pathogenicity of *L. monocytogenes* .

3. With the data gathered, derive a mathematical equation which could be applicable to the conditions used in this study. This model could then be used for predictive purposes to ensure/ assess safe handling of the product.

REVIEW OF LITERATURE

Microbial and Safety Implications of Ground Beef

Deterioration of whole, muscle meat results from a number microbiological and biochemical factors (Huis in't Veld, 1996). It has been long established that comminution accelerates the process of spoilage in fresh meat (Rogers and McCleskey, 1957; von Holy and Holzapfel, 1988). Contributing factors include: that integrity of the meat has been compromised from the grinding process therefore, it is more susceptable to microbial spoilage primarily through an increase in surface area; grinding allows for further penetration of initial surface contamination; and there is potential for added contamination during the grinding process. In addition, potentially pathogenic bacteria present in meat are not detected during veterinary inspection at the time of slaughter (Pivnick et al., 1976).

Epidemiological data indicate that adequately cooked beef has not been the cause of foodborne disease unless it has been mishandled or recontaminated after cooking (Anon. 1975). However, consumption of raw or grossly undercooked ground beef has resulted in outbreaks of toxoplasmosis (Lord et al., 1975), salmonellosis (Fleming et al., 1973) and hemorrhagic colitis (Doyle, 1991). Contamination of ground beef in the United States with *E. coli* O157:H7 caused 477 cases of severe hemorrhagic diarrhea, some of which progressed to the hemolytic uremic syndrome stage with three deaths (Thayer, 1995). Thus, eliminating the presence of pathogens and/ or controlling their growth during production and storage is key to extending

product shelf-life and assuring safety. Many organisms including pathogens, do not grow or grow very slowly in products that are adequately refrigerated (< 4^0C). Also, they are destroyed by cooking when the center of the meat is no longer pink; about 70^0C (Pivnick et al., 1976). Among the numerous parameters which affect both the lag phase and rate of growth of microorganisms is the temperature of the environment and composition of the atmosphere in which the food is kept (Genigeorgis, 1985). At the present, the proper control of these parameters is used extensively in extending the shelf-life of fresh muscle foods (Wolfe, 1980).

Shelf-life Extension

With consumer driven demands for less processed and more natural foods containing fewer additives, there is a growing emphasis on refrigeration as the primary means of restricting the growth of pathogens and spoilage microorganisms (Kirov, 1993). Some organisms like *Aeromonas* and *Listeria* are mesophiles but have the ability to grow at chill temperatures of 3 to 5^0C (Kirov et al., 1990; Beuchat, 1991). For example, *Aeromonas* species naturally present in foods showed a 10 to 1000-fold increase during 7 to 10 days storage at 5^0C (Callister and Agger, 1987; Berrang et al., 1989). Likewise, Kallander et al. (1991) observed that *Listeria* increased 1 log over a 13 d storage period at the same temperature. As well, van Garde and Woodburn (1987) stated that the normal temperature maintained in 20% of home refrigerators sometimes exceeded 10^0C. These observations not only impact on the prospect for foodborne illness to increase but also the need to develop methods for shelf-life

extension of products at temperatures slightly above freezing (Jaye and Ordal, 1962; von Holy and Holzapfel, 1988).

Modified Atmosphere Packaging Technology

Modified atmosphere packaging (MAP) has become an increasingly common approach to extend the shelf-life of perishable refrigerated foods (McDaniels et al., 1984; Rice, 1989; Ingham et al., 1990; Wimpfheimer et al., 1990). In this technology, food is packaged in high barrier packages in which air has been replaced with an artificial, modified atmosphere. The most common gases used include: oxygen, nitrogen, air, carbon dioxide, or more commonly, a mixture of two or more of these gases (Seideman and Durland, 1984). Nitrogen, an inert gas, is used as a filler to reduce the concentrations of the more active gases. Gases, like O_2, are used are to prevent color deterioration, while CO_2 is used to control spoilage bacteria such as *Pseudomonas, Acinetobacter* and *Moraxella* and mold. For poultry, packaging under high CO_2 atmospheres (20%) has been reported to extend the storage life approximately threefold over that attained for similar products stored in air (Hotchkiss et al., 1985; Sanders and Soo, 1978). When compared to air, CO_2 levels greater than 10% inhibit the growth of common spoilage bacteria, principally gram-negative rods (Baker et al., 1985) while allowing many gram-positive organisms to proliferate. In effect, development of off-odors, off-colors and spoilage that are associated with rapid growth of gram-negative psychrotrophs may be inhibited and therefore deterioration of the product is slowed (Wimpfheimer et al., 1990). This scenario, however, may play a

significant role in the frequency of foodborne illness because normal clues for food spoilage may be absent, despite the presence of sufficient numbers of pathogens to cause illness.

Theories regarding CO_2's role in the inhibition of microbial growth have been proposed. It is believed that CO_2 may affect cell membrane permeability (Enfors and Molin, 1979). Inhibition may be a result of CO_2 accumulation in the membrane of the lipid bilayer, thus increasing its fluidity. Labuza et al. (1992) proposed that one of the functions of CO_2 was to create carbonic acid on the food surface. Surface microorganisms would dissipate energy in an effort to maintain their internal pH, thus, resulting in retarding in growth.

With respect to aerobes, the efficacy of MAP is partially due to the fact that at reduced O_2 conditions or lack of O_2 conditions, these organisms are denied electron acceptors. This decreases their ability to grow or survive. In effect, CO_2 does not have an inhibitory effect on anaerobic spoilage or pathogenic organisms (Johnson et al., 1974).

Research has confirmed that a major concern with the use of MAP is whether facultative anaerobic pathogens, such as *L. monocytogenes*, *Staphylococcus aureus* and *Salmonella*, can grow to dangerous levels before nonpathogenic spoilage organisms, such as *Pseudomonas* signal spoilage by production of offensive odors and slime (Seideman and Durland, 1984; Daniels et al., 1985; Hintlian and Hotchkiss, 1987).

Influence of MAP at temperatures greater than 10^0C

Carbon dioxide at levels of 20% or more extend shelf-life by inhibiting the growth of many psychrotrophs (Baker et al., 1985; Hotchkiss et al., 1985). The inhibitory effect of CO_2 on microorganisms appears to be critically influenced by temperature (Knoche, 1980; Gould, 1996). Silliker and Wolfe (1980) found that 60% CO_2 significantly reduced the growth rate of salmonellae inoculated into ground beef, compared to samples in air at 10^0C. Their results also indicated that the effect of MAP on salmonellae growth at 20^0C was very slight. In addition, they demonstrated that staphylococci inoculated (10^4 CFU/g) into ground beef did not grow during storage at 10^0C; however at 20^0C, slow growth occurred during the first 60 hours of storage (from 10^4 to 10^7 CFU/g).

Enfors and Molin (1981) also reported that the inhibitory effects of CO_2 were temperature dependent. They studied the effect of 50% CO_2 on the growth of *Pseudomonas fragi* in muscle food and found that the growth rate was inhibited about 30% at 35^0C, 50% at 30^0C and, 90% at 5^0C. These researchers concluded that the inhibitory effect of CO_2 increased successively with decreasing temperature. This effect was explained by the increasing solubility of CO_2 with decreasing temperature (Knoche, 1980; Ogrydziak and Brown, 1982).

Baker et al. (1986) found that atmospheres containing elevated CO_2 levels reduced the growth rate of *Salmonella typhimurium* and *S. aureus* when inoculated into ground chicken over a temperature range from 2 to 13^0C. However, at 7 days, inoculated samples held at 7 and 13^0C contained microbial populations that

were too numerous to count regardless of whether the samples were packaged in 100% air or 80% CO_2. The authors noted that storage at temperatures of 13°C would be considered abusive for fresh chicken carcasses or parts. Temperatures which promoted pathogen growth in 80% CO_2 also promoted the growth of *Ps. fragi*.

In general, temperature had a greater influence on the growth and survival of microorganisms than did the composition of the atmosphere.

Irradiation Technology and Its Present Status

Irradiation is grouped into three categories based on the dose applied. Radappertization or commercial sterility requires doses greater than 20 kGy; radicidation or destruction of all non-spore forming organisms requires less than 10 kGy; and radurization or radiation pasteurization requires 10 kGy or less (Jay, 1970; Josephson and Peterson, 1983).

Therefore, depending upon the applied dose level, food irradiation has many uses in processing. For example, there is a thousand-fold difference between the dose required to inhibit sprouting of vegetables and potatoes (0.05 - 0.15 kGy), and that required to kill all microorganisms, that is, sterilization (25.0 - 50.0 kGy; Jones, 1992).

In Canada, food irradiation has been approved for some commodities since 1960 under the food additive regulations. In 1983, following the acceptance of the International Codex Standard, Health and Welfare Canada re-examined the existing

regulations for the application of food irradiation and proposed new regulations recognizing food irradiation as a process of food preservation (AIC/CIFST, 1989).

Effect of irradiation on the microflora present in food

With radurization, not all microorganisms present in a product are killed (Urbain, 1983). Doses of 2 to 3 kGy are sufficient to destroy gram-negative pathogens like *Salmonella*, *Campylobacter* and *E. coli* 0157:H7 (Ingram and Farkas, 1977; Loaharanu, 1995; Monk et al., 1995). However, some gram-positive organisms, like *Listeria*, are less sensitive to irradiation (El-Shenawy et al., 1989) and could survive treatment. During storage these survivors may multiply especially if the food was temperature abused. There are also concerns as to alterations in the character of the ultimate spoilage pattern in a way that the consumer may not recognize as spoilage (Urbain, 1983).

Grant and Patterson (1995) studied the thermal *D* values for *L. monocytogenes* CRA 433 in inoculated roast beef. The observed thermal *D* values for *L. monocytogenes* at 60, 65, and 70^{0}C in absence of pre-irradiation were 90.0, 53.0, and 28.0 minutes, respectively, whereas thermal *D* values after pre-irradiation were 46.4, 15.3, 7.8 minutes, respectively. A dose of 0.8 kGy caused a significant decrease in thermal *D* values, suggesting that irradiation treatment sensitizes *Listeria*. The persistence of the sensitizing effect was also observed after storage at 2 - 3^{0}C. *D* values obtained after irradiation and storage remained lower that those obtained for heating alone. These findings suggest that any listeriae surviving irradiation of a cook-

chill meal would have increased heat sensitivity throughout the refrigerated shelf-life of the cook-chill meat (Grant and Patterson, 1995).

Effect of MAP and Irradiation as Combined Treatments

Even though MAP can reduce growth of aerobic spoilage and pathogenic microorganisms, it does not generally kill them (Wimpfheimer et al., 1990). When CO_2-enriched atmospheres are removed from packaged foods (when the package is opened), an initial lag phase is observed before gram-negative bacteria begin to grow again. These microorganisms will ultimately spoil the product especially when stored at $< 5^0C$ (Silliker and Wolfe, 1980).

Irradiation doses from 1.75 to 5 kGy can extend product shelf-life when stored at chill temperatures from 6 to 14 days (Grant and Patterson, 1988). Also, it can greatly reduce the number of pathogens such as *Salmonella* and *Campylobacter* (Kampelmacher, 1983; Grant and Patterson, 1988). Grant and Patterson (1988) found that a dose of 1.75 kGy can significantly reduce the total bacterial count on commercial pork. However, not all microorganisms are destroyed at these doses (Thornley et al., 1960).

It has been found that the use of combination preservation treatments can be advantageous. Combined treatments principally allow for the less extreme use of any single treatment, with consequent improvement in product quality (Minaar et al., 1992). In this respect, many preservation systems are more effective when used in combination rather than singly (Gould, 1989). Therefore, combination treatments

have been proposed as a means of enhancing the preservative effect of irradiation (Vas, 1981). One potential combination protocol is the use of irradiation with MAP. Hastings et al. (1986) compared the irradiation resistance of lactobacilli isolates from beef irradiated in a normal atmosphere to beef irradiated under various atmospheres. The D_{10} values were found to be lowest when the isolates were irradiated under 100% CO_2, as compared to air, vacuum or N_2. This suggested that irradiation and CO_2 acted synergistically to enhance the lethal effect. In contrast, Patterson (1988) found that the irradiation sensitivities of *Streptococcus faecalis* and *S. aureus* were unaffected by the atmosphere in which they were packaged (100% air, 100% CO_2, 100% N_2 or vacuum). However, the authors reported that the D_{10} values of *Pseudomonas putida*, *Salmonella typhimurium*, *E. coli*, *Moraxella phenylpyruvica* and *Lactobacillus* species decreased when irradiated in atmospheres other than air. Patterson (1988) found that the presence of a CO_2 atmosphere during irradiation generally contributed a higher lethal effect as compared to air or N_2.

Presently, few studies have focused on the survival and growth of pathogens during storage in irradiated, modified atmosphere packaged food products under temperature abuse conditions. Grant and Patterson (1991b) stated that when high inoculum levels (10^6 CFU) of either *S. typhimurium*, *L. monocytogenes* or *E. coli* were used, the microbiological safety of irradiated pork packed in 25% CO_2: 75% N_2 and stored at abuse temperature of 10 or 15^0C was improved since all organisms were

significantly reduced. Furthermore, during storage, these pathogens were outgrown by lactic acid bacteria.

Incidence, Survival and Growth of *Listeria monocytogenes* in Foods

The genus *Listeria* contains seven species: *L. monocytogenes*, *L. seeligeri*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. grayi* and *L. murrayi* (Lovett, 1990). Of these seven species, *L. monocytogenes* is the principal pathogen in humans and animals. This organism is a mesophilic coccoid rod, with high salt toleranance and has a minimum growth temperature of approximately 2.5^{0}C. Therefore, it is also considered to be a psychrotroph. At 4 to 5^{0}C, *L. monocytogenes* has a doubling time in milk and cream of 1.5 to 2.0 days (Rosenow and Marth, 1987). In soymilk (Ferguson and Shelef, 1989) and eggs its doubling time is 1.6 and 1.7 days, respectively (Sionkowski and Shelef, 1989).

L. monocytogenes also has the ability to grow at a pH from 4.7 to 9.2 with a water activity (a_w) requirement of 0.92 or higher (Wehr, 1987). It is well documented that it exists and multiplies as a saprophytic organism in the soil and on plants as well as in sewage and river water (ANON, 1991; Farber and Peterkin, 1991; Wegener et al., 1993). In Denmark, *L. monocytogenes* has been isolated from cattle food items such as beets (31%), straw (29%), grain (23%), and hay (17%). Furthermore, it was present in 18% of 44 examined fecal samples in and around milking cows (ANON, 1991). Consequently, it is not surprising that bulk milk may

contain *L. monocytogenes*. In the USA, up to 12% of milk deliveries have been reported to be *L. monocytogenes* positive (Lovett et al., 1987).

This organism has also been detected in seafood, lettuce and is known to be associated with animal products (for example, beef and poultry) which are used for human consumption (Ingham, 1990). It has the ability to grow microaerophically between 2 - 45^{0}C (Shelef, 1989). *L. monocytogenes* also is a contaminant of cooked meat and poultry products (Nicholas, 1985; Ingham, 1990). In 1985, Nicholas isolated *L. monocytogenes* from 5 of 52 samples of frozen ground beef in France. Published reports from the USA and Europe have confirmed the presence of *L. monocytogenes* in approximately 25% and 47% samples of fresh meats and poultry, respectively (Shelef, 1989 and Bailey et al., 1989). Reported CFU's in raw meats ranged from less than 20 to 10^3 per gram. The ability of the bacterium to survive and grow in meat is particularly important since meats may be consumed after a brief heat treatment, which may not be sufficient to kill all viable cells (Karaioannoglou and Xenos, 1980).

Listeria is responsible for approximately 1700 cases of foodborne illness every year (ANON. 1989). The severity of listeriosis is evident in the fact that at least 54 of these cases resulted in death. Sporadic cases and epidemic outbreaks of listeriosis have been reported in humans and in various animal species (Farber and Peterkin, 1991; Rocourt, 1994). In the USA, an annual incidence rate of listerosis in the human population has been estimated at 0.7 cases per 100,000 population (Schuchat et al., 1991). The mechanisms by which *L. monocytogenes* causes listeriosis are poorly understood. The bacterium produces a series of toxins which may be

involved in the disease processes. Primary manifestations of the disease in humans are gastroenteritis, meningitis, abortion, and prenatal septicemia. Those individuals having the greatest risks for the development of the disease are pregnant women and newborns. Immunocompromised individuals with underlying illnesses such as malignancy and cirrhosis also are at substantially higher risk than healthy individuals (Wehr, 1987).

With the Food and Drug Administration and the Health Canada mandate of a zero-tolerance level for *L. monocytogenes* in ready-to-eat food products (Andrews et al., 1995), it is imperative that products be completely free of this bacterium upon reaching the retail market. Although the organism can be injured by exposure to a variety of food processing treatments including heating, freezing and exposure to acids or sanitizing compounds (Beuchat et al., 1986; Bunning et al., 1988; Golden et al., 1988), sublethally injured listeriae may be capable of repair in certain food products and therefore, possess the potential for pathogenicity. Meyer and Donnelly (1991) found that the ability of heat injured *L. monocytogenes* to undergo repair in pasteurized milk was sensitive to increases in temperature. For example, at 4^0C repair was completed after 16 to 19 days. However, this time was shortened to 4 days at 10^0C, and 13 and 9 h at 26 and 37^0C, respectively. Chawla et al. (1996) found that refrigeration was the most effective means to prevent the repair of heat injured *L. monocytogenes*. Conversly, they noted that exposure of injured *Listeria* to abusive temperatures enhanced repair.

Due to its ability to survive and proliferate under adverse conditions, like chill temperatures, and its ability to grow in microaerophilic environments (Wimpfheimer, 1990), the behavior of *L. monocytogenes* is of interest in refrigerated, extended shelf-life foods, such as those packaged with a controlled, or modified atmosphere.

Radiation sensitivity of *L. monocytogenes*

Due to the organism's ubiquitous nature, its ability to grow at chill temperatures, and its resistance to desiccation and freezing, WHO (1988) has suggested that irradiation could be used to eliminate *L. monocytogenes* from certain susceptible foods.

Studies have shown that 2.5 - 7.0 kGy is sufficient to eliminate listeriae (WHO, 1988; Stegeman, 1988; Patterson, 1989). Mead (1990) confirmed that *L. monocytogenes* is a common contaminant on raw chicken carcasses but showed that numbers are likely to be low immediately after processing and that they were largely destroyed by gamma irradiation using a dose of 2.5 kGy. Where survivors were found after irradiation, they either recovered slowly from sublethal injury or multiplied to detectable levels from small numbers of uninjured cells. These authors noted that *Listeria* seem to grow well on poultry skin at 5^0C. However, any multiplication before irradiation would reduce the chances of completely eliminating the organism from chilled carcasses (Mead et al., 1990). Tarjan (1990) found that some strains of *L. monocytogenes* could survive irradiation treatment as high as 4 kGy. In addition, cells which survived lower radiation doses (0.5 -2 kGy) could multiply during storage at

refrigerator temperatures. Furthermore, the results of Mead et al. (1990) did not support those of Huhtanen et al. (1989) which showed that 2.0 kGy was sufficient to destroy 10,000 CFU of *L. monocytogenes* on poultry.

Protective effect of beef tissue on *L. monocytogenes*

Mulder (1982) suggested that the protective effect of meat on the radiation response of microorganisms resulted from the presence of protein. Food components, such as proteins, are thought to compete with bacteria for interaction with radicals formed during the radiation process (Urbain, 1986). Therefore, the irradiation medium has a significant effect on the radiation resistance of the microorganism. For example, Patterson (1989) obtained lower D_{10} values for *L. monocytogenes* in phosphate-buffered saline as compared to poultry meat. El-Shenawy et al. (1989) and Mulder (1982) also found that the resistance of various strains of *L. monocytogenes* increased when the suspending medium was changed from saline to poultry meat. Mulder (1982) concluded that resistance of *L. monocytogenes* to radiation is dependent on the menstruum and strain. Generally, the bacterium is more resistant in beef than in broth. To date, no attempts have been made to determine the radiation resistance of *L. monocytogenes* in meat as influenced by tissue type (lean or fatty).

Pathogenesis and virulence of *L. monocytogenes*

The psychrotrophic properties of listeriae are of particular importance and have been studied in pathogenic and non-pathogenic strains (Junttila et al., 1988). In this respect, it was observed that virulent strains of *L. monocytogenes* grew at lower temperatures (0 to 6^0C), suggesting that they have a higher tolerance to lower

temperatures and may better survive harsh environmental conditions compared to other listeriae strains (Junttila et al., 1988).

All pathogenic strains of *Listeria*, given proper cultural conditions, are capable of producing varying amounts of hemolytic activity (Chakraborty and Goebel, 1988). The hemolytic activity obtained in vivo has been attributed to a single molecule, listeriolysin. Listeriolysins are proteins believed to be the principal virulence factors and since non-hemolytic strains are not virulent, they lack listeriolysins (Shelef, 1988). The exact function of listeriolysins during the infectious process is not well understood. It is thought to play a role in disruption of phagosomes of mononuclear phagocytes and in the release of intracellular iron during the infectious process (Wilder and Sword, 1967; Kingdon and Sword, 1970). In the latter situation, release of sequestered iron facilitates bacterial growth (Weinberg, 1974). Listeriolysin is toxic to both erythrocytes and leukocytes (Njoku-Obi et al., 1963). Also, hemolytic activity may enable the bacterium to survive the phagocytic process (Hof, 1984).

Attempts have been made to establish a correlation between hemolysin production and *L. monocytogenes* virulence. However, Hof (1984) reported that the hemolytic activity of a number of *Listeria* strains failed to show a correlation with virulence. Drastically reduced hemolytic activity was observed after purification of crude hemolysin, suggesting that there may be more than one hemolysin present (Jenkins et al., 1964).

The loss of hemolysin production is followed by a total absence of pathogenicity (Hunter et al., 1950; Hof, 1984); this can occur spontaneously (Pine et

al., 1987) or can be induced by irradiation (Hunter et al., 1950) or by genetic engineering (Goebel et al., 1988). Non-hemolytic phenotypes may be induced either by blockade of the hemolysin gene itself or by a functional abrogation of a promoter region regulating the hemolysin gene activity (Leimeister-Wachter et al., 1989).

Leimeister et al. (1992) reported that the pathogenic factors in *L. monocytogenes* are dependent on growth temperature. The expression of listeriolysin is positively regulated by a transcriptional activator, the PrfA gene product. Mutation of virulence genes are positively regulated at the transcriptional level by the production of the PrfA gene. These researchers observed that the pattern of transcription of PrfA was more complex at 37^0C than at 20^0C.

Mekalanos (1992) and Datta (1994) found that virulence/pathogenicity of *L. monocytogenes* was affected by various substrate factors. Furthermore, Buncic et al. (1996) observed that storage of two *L. monocytogenes* strains (NCTC 7973 and a food isolate) under conditions that prevented their growth (that is, in nutrient free substrate at refrigeration temperature) resulted in attenuated pathogenicity as well as in an extended lag phase when moved to 37^0C. However, pathogenicity and growth characteristics of a clinical strain, used in the same study, were less affected. Avery and Buncic (1997) observed that clinical strains of *L. monocytogenes* maintained pathogenicity significantly longer than food strains when exposed to unfavourable storage conditions (4^0C). It has been hypothesized that resistance of certain strains of *L. monocytogenes* to negative factors acting in foods may contribute to the particular capability of certain strains to cause illness and consequently, become clinical strains

(Avery and Buncic, 1997). This is supported by findings that stress, acid, or stationary phase growth induced cellular mechanisms that significantly affect virulence of some strains (Hill et al., 1995; Rees et al., 1995; Archer, 1996).

To date, no studies have been reported on the effect of environmental conditions, such as temperature and/or CO_2 in conjunction with irradiation, on the virulence of *L. monocytogenes* and its pathogenesis in animals.

Mathematical Modeling and Its Application in Microbiology

Currently, there is considerable interest in modeling the effects of different parameters on the growth of microorganisms to predict the shelf-life and safety of foods (Baird-Parker and Kilsby, 1987; Gould, 1989, Roberts, 1990). In food microbiology, mathematical modeling is an area where models are used to describe the behavior of microorganisms under different physical and chemical conditions. Quantitative models are used to design and interpret the results of microbial experiments. In some areas of food microbiology, it has been standard practice to make use of mathematical models (Gould, 1989). An example is the thermal death time model for the destruction of *Clostridium botulinum* spores during heating of low acid canned foods. Roberts and Jarvis (1983) considered mathematical modelling to involve measuring the growth responses of this organism to factors influencing the rate and type of microbial growth in food and from those data, attempting to predict what will happen during storage.

Many extrinsic and intrinsic factores, including pH, water activity (A_w), nutrient content, antimicrobial constituents including competitive organisms, and temperature have been shown to affect the rate and the extent of microbial growth in foods (Labuza et al., 1992). These factors may act singly and/or have synergistic effects. Of the many factors that influence the rate of change of microbial numbers in foods, pH, A_w, and temperature are particularly important in mathematical modelling (Christian and Waltho, 1962; Roberts and Jarvis, 1983).

Gould (1989) suggested that although modeling of microbial growth and survival is applicable to all types of microorganisms and to some extent to parasites and viruses, primary concentration should be focused on food related microorganisms that are of major public health significance with most efforts directed to *Salmonella typhimurium*, *S.enteritidis*, *L. monocytogenes*, *Clostridium* and *Bacillus* species. The Microbial Food Safety Research Unit of the United States spent approximately US$500 000 in a five year program on studying the effect of temperature, NaCl concentration, pH, nitrite concentration and gaseous atmosphere on *L. monocytogenes* (McMeekin et al, 1993). This level of research expenditure is the result of the enormous cost of food poisoning outbreaks

Arrhenius Law

The Arrhenius equation describes the effect of temperature on the rate of chemical reaction (Labuza et al., 1992). It has been successfully used in describing the temperature dependence of many simple and complex chemical reactions. Since microbial growth is a complex set of enzyme-mediated biochemical reactions, it can

also be characterized in terms of overall activation energy if all ecological factors are kept constant (Labuza et al., 1992). The Arrhenius relationship models the effect of temperature on growth by (Labuza et al., 1992):

$$\ln\left(\frac{S}{S_o}\right) = kt = k_o \exp\left(\frac{-E_A}{RT}\right)t \qquad (1)$$

where, S = the number of microorganisms (CFU/ml) found after time t

S_0 = initial population

k = specific growth rate of bacteria over a limited temperature range

k_0 = "collision" or "frequency" factor

T = the absolute temperature in K

R = universal gas constant (8.314 J/mol)

E_A = activation energy (J/mol).

Equation 1 is generally known as the Arrhenius Law (Ratkowsky et al., 1982).

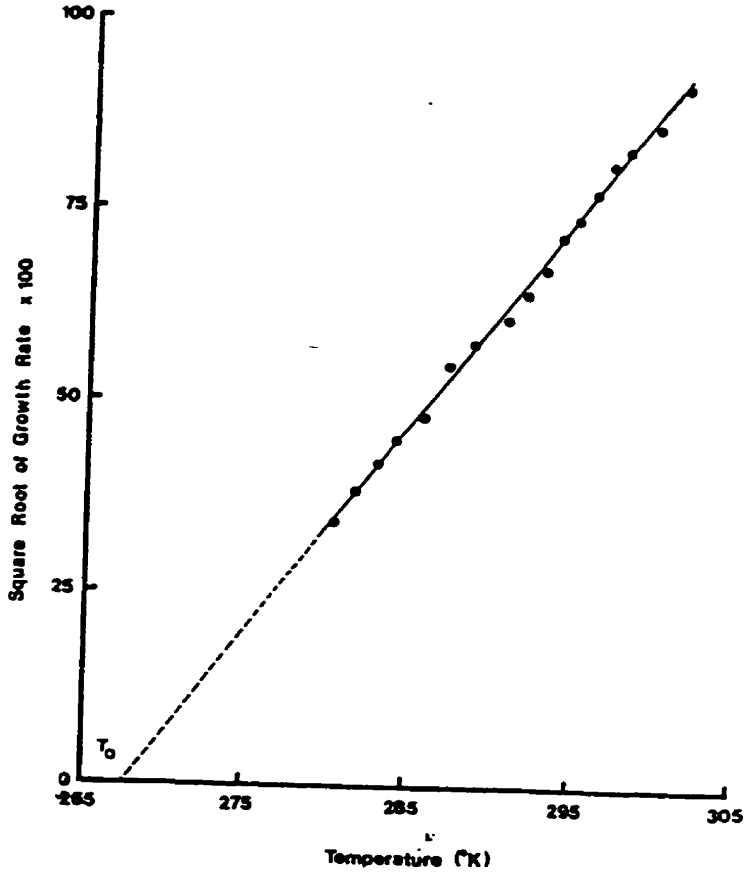
The specific growth rate constant, k, is an index of the growth rate for a particular organisms (McMeekin and Olley, 1986). It has a maximum value at the optimum temperature of growth for the organism and is zero at temperatures that are greater than the maximum or less than the minimum temperature of growth. The E_A is a measure of the temperature sensitivity of the growth rate dependent reaction. The higher the E_A, the greater the increase in growth rate for an increase in temperature. This value for microbial growth ranges from 60-120 kJ/mol (Labuza et al., 1992).

The Arrhenius Law states that a plot of the log of the growth rate constant versus the reciprocal of the absolute temperature will give a straight line, as seen in Figure 1. This model can be used to predict the growth rate of an organism for any temperature condition as long as it is within the upper and lower limits of the temperature range used to create the data set.

Since bacterial growth is an interaction of a complex set of reactions involving both catabolic and anabolic processes, Arrhenius plots of specific growth rates may deviate from linearity. As a consequence, when a plot of the log of the growth rate constant against the reciprocal of the absolute temperature is made, the curve which results is concave downward towards the 1/T axis instead of a straight line (Johnson et al., 1974; Ratkowsky et al., 1982).

Bacterial growth is a complex biological process involving a variety of substrate and enzymes, thus the Arrhenius Law does not adequately describe the effect of the temperature on growth of bacteria (Ratkosky et al., 1982). Within a microbial cell, there are many enzymatic and metabolic changes. Since the Arrhenius equation was originally formulated to describe single step chemical reactions, it does not adequately describe the relationship between temperature and bacterial growth. Also, as the reaction mechanisms change in the cell as the result of temperature change, the E_A may also vary (Ratkowsky et al., 1982). The E_A energy may vary as much as three- of four-fold throughout a single set of data depending upon which portion of the data set is used.

Figure 1. Arrhenius model plot for *Ps. fragi* growth rates in the range of 2 to 22^{0}C. Data taken from Labuza et al., 1992.


100
75
50
25
0
Square Root of Growth Rate x 100
T_0
265
275
285
295
305
Temperature (°K)

Square Root Model

Ratkowsky et al. (1982) refined the Arrhenius equation to more accurately reflect bacterial growth; this refinement resulted in an equation relating temperature to the square root of growth rate. The equation is:

$$\sqrt{k} = b\,(T - T_{min}) \qquad (2)$$

where, k = the specific growth rate constant over a limited temperature range

b = the coefficient found from the slope of the regression line

T = the temperature in K

T_{min} = a conceptual temperature below which no growth can occur

As shown, this model demonstrates the temperature dependence of the specific growth rate constant at temperatures between the minimum and the optimum temperatures for growth of the organism. One of the advantages of the square root model is that it provides a good linear fit to experimental data.

This relationship was found to apply to data for 43 strains of bacteria grown at temperatures ranging from their minimum to just below their optimum temperature (Ratkowsky et al., 1983). One of the advantages of the square root model is that it provides a good linear fit to experimental data. As shown in Figure 2, the T_{min} value is derived by extrapolating the regression line to the temperature axis; the value of T when $\sqrt{k} = 0$, by regression. T_{min} is not the minimum temperature for growth of

the microorganisms; it appears to be an intrinsic property of the organism (Chandler and McMeekin, 1985). T_{min} is usually 2-3^{0}C lower that the temperture at which growth is actually observed. It has been described as the conceptual temperature or the "biological zero" at the low end of the temperature range (McMeekin et al., 1993). T_{min} may be used as a basis for the categorization of bacterial as psychrophiles, mesophiles or thermophiles, as seen in Table 1.

Figure 2. Square root of relative growth rate as a function of temperature for psychrotrophic spoilage ($T_{min} = -10^0C$). Data taken from McMeekin and Olley (1986).

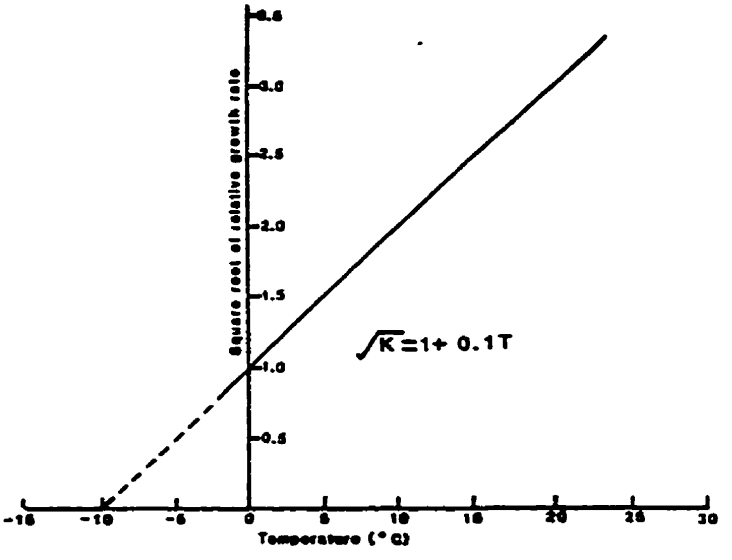

Square root of relative growth rate
3.5
3.0
2.5
2.0
1.5
1.0
0.5
$\sqrt{K}=1+0.1T$
-15
-10
-5
0
5
10
15
20
25
30
Temperature (° C)

Table 1. T_{min} values for various bacteria.[a]

Classification	Culture	T_{min} (K)
PSYCHROPHILE	*Pseudmonas* sp. L12	248
PSYCHROTROPHS	*Pseudomonas* sp. Gp IV	263.5
	Pseudomonas fluorescens	264
MESOPHILES	*Proteus morganii*	272
	Escherichia coli	275
	Pseudomonas aeruginosa	276
	Acinetobacter sp.	277
THERMOPHILES	*Lactobacillus delbrueckii*	290
	Bacillus circulans	296

[a]Data taken from Chandler and McMeekin, 1985.

T_{min} is a hypothetical concept since the Square Root Model is valid only at temperatures where water activity is not changing due to ice formation (Ratkowsky et al., 1982). Ratkowsky et al. (1982) stated that this value is an intrinsic property of the organism when growth conditions, other than temperature, are non-limiting. For psychrotrophic spoilage, the T_{min} value is 263 K (approximately -10^{0}C). In Figure 3, a comparison of rates at any temperature T and 0^{0}C can be made. From Equation 2:

$$\left(\frac{k}{k_o}\right)^{\frac{1}{2}} = \frac{(T-263)}{(273-263)} \qquad (3)$$

where, k_0 is the specific growth rate at 0^{0}C.

Using temperatures in degrees celcius instead of Kelvin, Equation 3 can be rewritten as (McMeekin and Olley, 1986):

$$\sqrt{k} = 1 + 0.1T(^0C) \qquad (4)$$

Therefore, if the rate at 0^{0}C is taken to be 1, the relative square root rate at 10^{0}C = 2 (relative rate = 4) and at 20^{0}C = 3 (relative rate = 9), as seen in Figure 3. The basic principle of relative spoilage rates can also be applied to mesophiles, including organisms of public health significance, like *E. coli* and coliforms.

However, at higher temperatures, Equation 2 ceases to model growth adequately due to the inactivation or denaturation of proteins, the increasing influence of mesophilic flora and other factors (McMeekin and Olley, 1986). In this respect, the psychrotrophic relative curve works well at temperatures up to 15 to 20^{0}C, however, at elevated temperatures, discrepancies are found (McMeekin and Olley, 1986).

Expanded Square Root Model

The optimum temperature for growth of mesophilic organisms like *E. coli* and *S. typhimurium*, which are of health concern, is usually in the region of 30 - 37^{0}C. However, considerable growth may also occur up to 45^{0}C. This temperature lies in the area between the optimum and maximum temperature of growth. The square root model describes the effect of temperature between the minimum and the optimum range. However, the specific growth rate declines quite markedly at suboptimal temperatures since heat denaturation of cell proteins can occur and death may result. Thus, to describe the temperature dependence of the growth rate in the optimum and the maximum temperature regions, the equation has been extended as follows (McMeekin and Olley, 1986):

$$\sqrt{k} = b(T-T_{min})\{1 - \exp [c(T-T_{max})]\} \qquad (5)$$

where k, b, T, and T_{min} are the same as Equation 2

c is the slope above the optimum, and

T_{max} (^{0}C) is the maximum temperature at which growth is observed

This model describes the growth rate of an organism around the optimum and the maximum temperature (Zwietering et al., 1991). When T is much lower than T_{max}, the term in the braces, {}, is negligible and Equation 5 becomes the Square Root Model (Equation 2). As T increases and approaches T_{max}, the term in the braces becomes more important until it dominates and the growth rate falls as T exceeds the optimum temperature, reaching zero when T= T_{max}.

The parameters *b* and *c* have no interpretable meaning. They present to enable the model to fit the data for temperatures above the optimal temperature. The Expanded Square Root Model has been successfully applied to data for 29 strains of bacteria (McMeekin et al., 1993).

Both T_{min} and T_{max} occur at points where the square root plot intersects the temperature axis at $\sqrt{k} = 0$ (at both points where the growth rate is zero). Just as T_{min} is the 'biological zero' at the low end of the temperature range, T_{max} is the 'biological zero' of the high end of the temperature range (McMeekin et al., 1993). Since it is very difficult to obtain accurate data at very low growth rates, the T_{min} and T_{max} may not be true temperature limits. Thus, this is a disadvantage of the Square Root and the Expanded Square Root Model. Like the Square Root Model, the predictive value of the Expanded Square Root Model is guaranteed only at a constant temperature within the temperature range of bacterial growth (van Impe et al., 1992).

Additionally, freezing alters water activity and affects the growth rate causing the curve to deviate from linearity. Thus, the actual minimum temperature of growth may differ from the extrapolated T_{min} value. For example, the calculated T_{min} is 3.5^0C for *E. coli*, but the experimentally determined minimum temperature for growth of this organism is 8^0C (Labuza et al., 1992).

Gompertz Function

The Gompertz Function is a quantitative model used to describe growth kinetics. It has been shown to empirically model microbial growth curves with

reasonable accuracy and to produce statistically interpretable summaries (Gibson et al., 1988). Researchers have used the Gompertz Function as a measure of statistically comparing growth parameters. Buchanan et al. (1989) utilized the Gompertz Function to model the effects of growth medium composition (e.g. salt concentration, pH, temperature, etc.) on growth of *L. monocytogenes*. In addition, Palumbo et al. (1991) studied the influence of temperature, pH, sodium chloride, and sodium nitrite on the growth kinetics of *Aeromonas hydrophila*. They obtained values for the lag and generation times as influenced by these conditions. These investigators were able to use the Gompertz model to provide estimates of the growth rates of *A. hydrophila* as a result of change in nutrient composition and storage conditions.

Chawla et al. (1996) used the Gompertz equation in combination with the first order model to effectively estimate the repair time from which the impacts of envirnomental conditions on the repair of heat injured *L. monocytogenes* could be quantitatively defined. The Gompertz parameters, which were generated by fitting the equation with the bacterial counts, were used to calculate the repair percentage as a function of time from which the repair time was estimated. These researchers found that all growth curves fit the Grompertz equation well ($R^2 \geq 0.972$).

The Gompertz Equation is:

$$\log_{10}CFU = A + C \exp\{-\exp[-B(t-M)]\} \qquad (6)$$

where, A = asymptotic log count of bacteria as time (t) decreases indefinitely (initial level of bacteria, $\log_{10}$ [CFU/ml])

C = asymptotic amount of growth that occurs as t increases indefinitely (number of log cycles of growth, $\log_{10}$ [CFU/ml])

B = relative growth rate at M, ($\log_{10}$[CFU/ml])

M = the time at which the absolute growth rate is maximal (h).

In order to build models to describe the growth of microorganisms in food, researchers normally first construct growth curves (Zwietering et al., 1990). To reduce measured data to parameters such as growth rates, it has been recommended that the data be described with a model instead of by using linear regression over a subset of data (Zwietering et al., 1990). Sigmoidal models to describe the growth data can be constructed with three or four biological parameters. Thus, Zwietering et al. (1990) compared several sigmoidal functions containing either three or four parameters to describe the bacterial growth curve. These models were the logistic, Gompertz, Richards, Schnute and the Stannard Models. These models describe only the microbial growth and do not include the consumption of substrate. The substrate level was not considered important since it was assumed that it was present in excess to reach large numbers of organisms.

Zwietering et al. (1990) reported that the Gompertz model best described growth data when compared to other models. It was able to give reasonably good fits of the data; the Gompertz Function was statistically sufficient to describe the growth data of *Lactobacillus plantarum*. It was accepted in all cases by the t test and was accepted in 95% of the cases by the F test. Zwietering et al. (1990) recommended that the Gompertz Function be used over other models because it was easy to use. In

addition, the estimates have more degrees of freedom, which can be important when a growth curve is used with a small number of measured points. Also, all the parameters in the Gompertz Function can be given a biological meaning.

One limitation recognized by Zwietering et al., (1990) is that the predictive value of this model can be guaranteed only at a constant temperature within the temperature range of microbial growth. In practice, however, the food product may be subjected to wide temperature variations. This hampers the application of the Gompertz Function in process design and control (Zwietering et al., 1990).

Since the effectiveness of a model is ultimately dependent on its ability to predict "real world" data, Buchanan and Phillips (1990) reported that the Gompertz Function is generally conservative in that it tends to overestimate the ability of the organism to grow under adverse conditions. The researchers believe this may be the result of additional factors not reflected in the model that influence the growth of the microorganism in the food systems.

Observations were made of the Gompertz model in its ability to describe the growth of *L. monocytogenes* (Farber et al., 1996). It was found that this model was "fail-safe" in terms of the predicted lag phase. That is, the model predicted lag phases that were shorter than the fitted values. For generation time, however, model predictions were not always "fail-safe", but were, nevertheless, very close to the fitted values .

Overall it is believed that the model appears to provide reasonable "first round estimates" that should be very useful in terms of allowing food microbiologists

and individuals involved in new product development to assess quickly the impact of altering any combination of the variables (Buchanan and Phillips, 1990).

MATERIALS AND METHODS

MATERIALS

Organism and Maintenance

Listeria monocytogenes (No. 10-112) was obtained from the Cadham Provincal Laboratory (Winnipeg, Manitoba). The organism was maintained on trypticase soy agar (TSA, BBL) slants at 4^0C following growth at 35^0C for 48h. On a monthly basis, the organism was transferred to freshly prepared TSA slants.

Ground Beef

Bulk packaged (styrofoam base, clear overwrap) ground beef was purchased from a retail outlet. The beef was labelled as regular, lean, or extra lean. Eye of round roast, purchased and mechanically ground at the retail outlet, was used to make extra-extra lean.

(i) moisture content

The moisture content (2 - 3g, dry weight) was determined using an oven drying procedure (18h) as described by Joslyn (1970). Results are expressed as means of triplicate values.

(ii) lipid content

Crude fat content (3 - 4g, dry weight) was determined by Soxlet extraction (16h) using hexanol as described by Joslyn (1970). Results are expressed as means of triplicate values.

(iii) protein content

The protein content (30 - 40 mg, dry weight) was determined by micro-Kjeldahl as described by Joslyn (1970). Results are expressed as means of triplicate values.

METHODS

Sterilization

Sterilization of all equipment and media was attained by autoclaving for 15 min at 121^{0}C.

Listeriae preparation

A loopful of *L. monocytogenes* (maintained on TSA slants) was inoculated into trypticase soy broth (25 ml) and incubated at 35^{0}C for approximately 18 h. Portions of the resultant growth (10 ml) were added to a flask containing fresh TSB (75 ml). The contents were gently agitated and transmittance readings (420nm) were taken using a Bausch and Lomb (Spectronic 20) spectrophotometer. Cultures with readings between 85 - 95% transmittance corresponding to 10^6 CFU/ ml were used. (Fresh sterilized TSB was used to standardize the inocula). This relationship was previously confirmed using a standard curve in which plate counts were related to transmission. In this regard, organisms were serially diluted in 0.85% NaCl and plated on TSA (24h, 37^{0}C). Uninoculated but sterilized TSB was used as a blank to standardize the spectrophotometer.

From the TSB containing 10^7 CFU/ ml of listeriae, 10 ml was taken and added into 90 ml of 0.85% NaCl. This resulted in 10^6 CFU/ ml of which 1.0 ml was be inoculated into 10 g portions of ground beef.

Sample preparation for irradiation

Portions (10g) of ground beef were weighed into in Surevak Paxe 2050 bags (Winpak, Winnipeg; 18.5 x 21.5 cm). The oxygen and vapor transmission rates of the bags were 8-10 $cm^3/m^2/24h$ and 4.96 $g/m^2/24$ h at 37.8^{0}C, 90% R.H., respectively (Information provided by Winpak). The bags were then folded over, taped shut and frozen at -20^{0}C.

Sample sterilization using irradiation

The frozen samples were irradiated using a I-10/1, 10 Mev electron accelerator housed at Atomic Energy of Canada, Limited, Pinawa, Manitoba. In this regard, the sample packages were placed on top of 3 to 4.5 cm styrofoam trays located in the bottom of aluminum trays. The bags were aligned in 2 to 3 rows, without overlapping; each row was no greater than 12 cm from the longitudinal centre line of the accelerator to ensure even dose application. The time of irradiation under the beam was approximately 20-25 sec at 25 kGy (sterilizing dose) with a dose rate at ca. 1 kGy/s.

Actual doses received by the samples were determined using radiochromic GAF dosimeters (GAF, Miller and McLaughlin, 1981). These dosimeters were used when samples were exposed to dosages less than 3 kGy. FWT dosimeters were used when

samples were exposed to doses greater than 3 kGy. Absorbed doses were determined by AECL dosimetry section personnel.

Inoculation of *L. monocytogenes* into samples

Following product sterilization, the samples were opened in a laminar flow hood and inoculated with *L. monocytogenes* (0.1 ml; 10^6 CFU/ml). The bags were then retaped and the contents were gently massaged by hand for ca. 30 seconds in order to distribute the inoculum. Controls consisted of irradiated but non-inoculated meat samples. These were used to assess the efficacy of the sterilization process.

Packaging equipment

A Bizerba (model 2002) packaging machine was used for the modified atmosphere packaging (MAP) studies. The desired gas mixtures (backflush once via a pressure regulating valve) consisted of either 15:85% CO_2:N_2 or 30:70% CO_2:N_2 and were obtained from a commercial source (Union Carbide Gas, Winnipeg). The backflush gas was supplied at approximately 13 psi.

All packaging operations were carried out at room temperature (ca. 21-22^{0}C). For MAP, the bags were opened and the ends were placed over the gas flush nozzle on the vacuum packaging machine. The settings on the machine were: vacuum: 50; gas flush: 10; and heat seal: 5. The vacuum packaging machine sealed the bags approximately 1.2 cm from the edge.

Samples were also packaged without a CO_2 backflush (inoculated control) and similarly sealed.

Sample irradiation at 1.75 kGy

Irradiation of MAP samples and inoculated controls were performed using a linear accelerator (Impela I-10/1, 10 Mev, AECL, Pinawa, Manitoba). The time of irradiation under the beam was ca.10 sec. Three dosimeters were randomly taped to the top of the treatment bags in order to verify the applied dose. In all cases, this dose was 1.75 kGy $\pm$ 10%. Following treatment, the samples were placed in coolers with icepacks for transport to the laboratory. Samples were subsequently stored either at 5^0C or at 11^0C in thermostatically controlled refrigeration units.

Microbiological analysis

Testing of samples at day 0 was carried out within 6 h of packaging. At specified time periods (7, 14, 21d, etc.) samples were removed from their storage environment and microbiologically examined. In this regard, the contents of each bag were stomached (90 ml; 0.1% peptone) for approximately 60 sec (model 400 Stomacher), serially diluted (0.1% peptone) and pour plated using TSA. CFU's were evaluated following incubation at 35^0C for 48 h. Results are expressed as means of triplicate samples.

Preparation of samples for assessment of hemolytic activity and pathogenicity

At day 0 and every 7 d interval, ground beef samples were removed from their storage environment of 5^0C or 11^0C.

The method for the preparation of samples regarding assessment of hemolytic activity was outlined by Brackett and Beuchat (1990). Potassium phosphate

buffer (0.1M, pH 7.0, 20 ml) was combined with samples of packaged beef (47) (10g) and gently hand massaged for 1 minute. The contents of each bag were then filtered through sterile glass wool filters (glass wool contained in a 15 cm length glass funnel having a 2.5 cm diameter at one end and a constricted outlet (ca. 1 mm). The filtrate, collected in 25 ml sterile centrifuge tubes, was centrifuged (7,500 x g for 10 min at 25^{0}C) and the supernatant decanted. The pellet was suspended in 5 ml of sterile 0.1M potassium phosphate buffer (pH 7.0) containing 10% sterile glycerol and stored at -18^{0}C. Uninoculated ground beef (47) samples were similarly prepared.

Confirmation of hemolytic activity

The frozen pellets were quickly thawed in a water bath (20^{0}C) and 0.1 ml samples were surface plated onto modified McBride agar (Blanco et al., 1989) and incubated at 37^{0}C for 48 h. Following growth, an overlay (8 ml) was added to each plate. The overlay consisted of (1 litre): red sheep blood cells (50 ml; Department of Animal Science, University of Manitoba), BHI broth (37 ml), agar (3 g), and NaCl (8 g), as outlined by Blanco et al. (1989) and was used to detect haemolytic activity. The overlayed plates were incubated for 14 h at 30^0 C and haemolysis was recorded qualitatively. Haemolysis was characterized by a distinct clear zone surrounding the organism in the red cell layer (overlay) background (Blanco et al., 1989). Control samples consisted of uninoculated agar plates to which the red sheep blood cells layer were added. Uninoculated ground beef (47) samples were similarly evaluated.

Pathogenicity evaluation

In order to evaluate the pathogenicity of *L. monocytogenes* in MAP stored ground meat, a chick embryo test as described by Terplan and Steinmeyer (1989) was utilized. In this test, one ml portions, obtained from the frozen pellets (quickly thawed at 20^{0}C in a water bath) were inoculated into the chorioallantoic membrane of 10 d chick embryos (Department of Animal Science, University of Manitoba) via a small opening of the blunt end of the egg using a disposable, sterile syringe (1 ml; Monoject, St. Louis, MO). Directly after inoculation, the opening was sealed using candle wax. The eggs were incubated in the vertical position in a Robbins Incubator with the dry bulb operating temperature of 100 ^{0}F. In order to provide additional sensitivity to the testing regime, dilutions made from the frozen pellets were also evaluated. In this case, phosphate buffered saline was used as the diluent. In all cases the listeriae population (that is the population contained within the 1 ml portions used as inocula) were evaluated using a surface spread method with TSA (35^{0}C, 48 h). Results are expressed as means of triplicate samples.

For each storage treatment (15%, 30% CO_2, air; 5 and 11^{0}C) ten chick embryos were inoculated and vitality was monitored daily for 6 d, by transillumination (Caswell Egg Candling Lamp). Vitality assessment consisted of observing the blood vessels and embryo movement. The total mortality was recorded during the 6-day post inoculation period.

RESULTS

Proximate Analysis of Ground Beef

The proximate analyses for protein, fat and moisture content in the ground beef samples are shown in Table 2. For simplicity and based on protein content, regular ground beef was termed beef (20), lean ground beef was termed beef (47), extra lean ground beef was beef (50) and extra-extra lean ground beef was termed beef (54). The latter product was formulated to provide a greater range in protein content.

Inoculation Pack Studies of *L. monocytogenes*

The ground beef samples (20), (47), (50) and (54) were radurized at approximately 25 kGy in order to render them commercially sterile. *L. monocytogenes* was subsequently inoculated into the samples and the effect of various CO_2 concentrations (air, 15% and 30%) and storage temperatures (refrigerated, 5^0C and abused, 11^0C) on the growth of *L. monocytogenes* was investigated. In some trials, the samples following inoculation were radiated at 1.75 kGy and the aforementioned storage protocol repeated. This procedure was performed in order to evaluate the growth and/or survival of listeriae using a combination treatment.

Table 2. Proximate analysis of ground beef samples.

Type	Moisture[1]	Fat[1]	Protein[1]
Regular (beef (20))	41.4 ± 2.0[2]	30.2 ± 3.6	20.0 ± 1.8
Lean (beef (47))	29.2 ± 0.4	19.8 ± 0.1	47.0 ± 0.1
Extra Lean (beef (50))	28.2 ± 0.5	17.8 ± 1.4	49.7 ± 3.0
Extra Extra Lean (beef (54))	27.3 ± 0.5	12.8 ± 2.8	53.9 ± 2.8

[1]%; Fresh Weight Basis (% F.W.B.)
[2]Values Represent Means ± SD; n=3

Survival and/or growth of *L. monocytogenes* in beef (20)

The survival of *L. monocytogenes* in packaged beef (20) containing either 15:85 % CO_2: N_2 (MAP) or air is presented in Figure 3. Maintained at 5^0C with MAP, the population appeared relatively constant at approximately 10^5 CFU/g throughout the 21 d storage period. In contrast, storage at 5^0C in air resulted in an increase in population from 10^5 to 10^6 CFU/g. Elevation of the storage temperature to 11^0C resulted in a sharp increase in the population between 0 and 7 d from 10^5 to 10^8 CFU/g or to 10^7 CFU/g when stored in CO_2 and air, respectively. The presence of CO_2 at the higher temperature appeared to have minimal inhibitory effect when compared to beef (20) packaged in air.

For all inoculated packs, irradiation (1.75 kGy) reduced the initial population from 10^5 to 10^2 CFU/g at (day 0), as shown in Figure 4. At 5^0C, the listeriae population remained constant at approximately 10^2 CFU/g until day 21 of storage when packaged in either 15% CO_2 or air. Thereafter, the counts decreased especially for the ground beef stored in CO_2. Elevation of the storage temperature to 11^0C resulted in a gradual increase in listeriae. At 28 d of storage, the population reached 10^8 and 10^7 CFU/g when stored in CO_2 and air, respectively. Inclusion of CO_2 in packs stored at 11^0C appeared to have a minimal inhibitory effect when compared to packs stored at similar temperature but without CO_2.

The growth of *L. monocytogenes* in ground beef stored either under 30:85% CO_2:N_2 or air is presented in Figure 5. At 5^0C in the presence of CO_2, the population appeared relatively constant at 10^5 CFU/g up until day 21 of storage;

thereafter the population increased ca 1 log by day 28 of storage. In beef packaged with air, a final population of ca. 10^6 CFU/g was obtained at day 28 of storage. Elevation of the storage temperature to 11^0C resulted in similar time course growth curves for listeriae, regardless of the packaging atmosphere. Final populations at day 21 of storage were ca. 10^8 CFU/g.

The initial population of listeriae (10^5 CFU/g) decreased to 10^2 CFU/g following the application of 1.75 kGy as shown in Figure 6. At 5^0C in the presence of 30% CO_2, the population appeared to remain static at ca. 10^2 CFU/g throughout the 42 day storage period. However, in air the listeriae population increased; at 42 d storage it reached 10^5 CFU/g. Increasing the storage temperature to 11^0C resulted in a rapid increase in the listeriae population stored in the presence of air. In ground beef, exposed to 30% CO_2 the population also increased but only after 14 d of storage.

Figure 3. Survival and/or growth of *L. monocytogenes* in packaged beef (20) containing either 15%:85 % CO_2:N_2 or air. Bars represent the SD of mean; n = 9. Appendix table: 1.

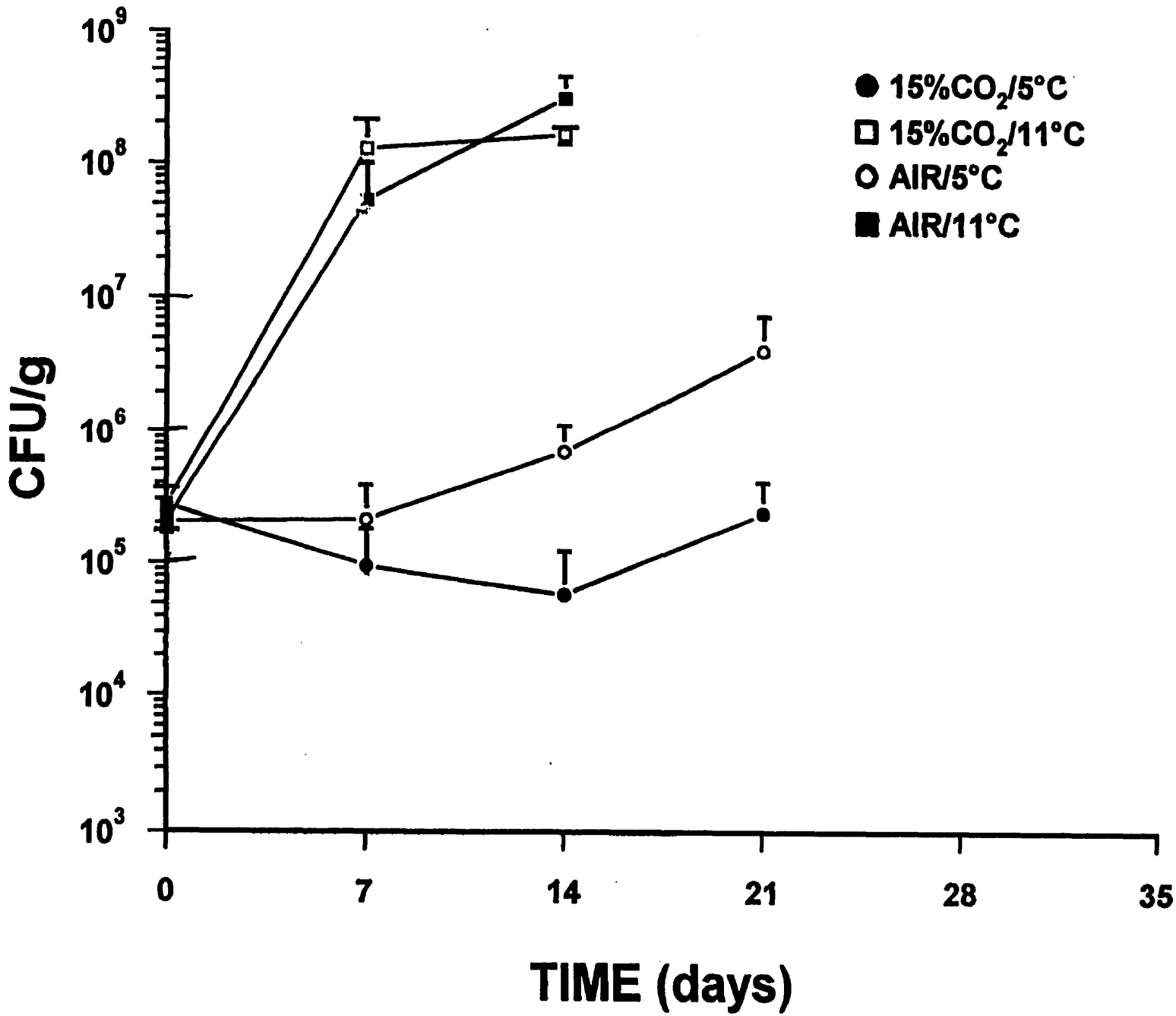

10^9
10^8
10^7
10^6
10^5
10^4
10^3
CFU/g
0
7
14
21
28
35
TIME (days)
15%CO_2/5°C
15%CO_2/11°C
AIR/5°C
AIR/11°C

Figure 4. Survival and/or growth of *L. monocytogenes* in packaged beef (20) containing either 15%:85% CO_2:N_2 or air follow by irradiation at 1.75 kGy. Bars represent the SD of the mean; n = 9. Appendix table: 2.

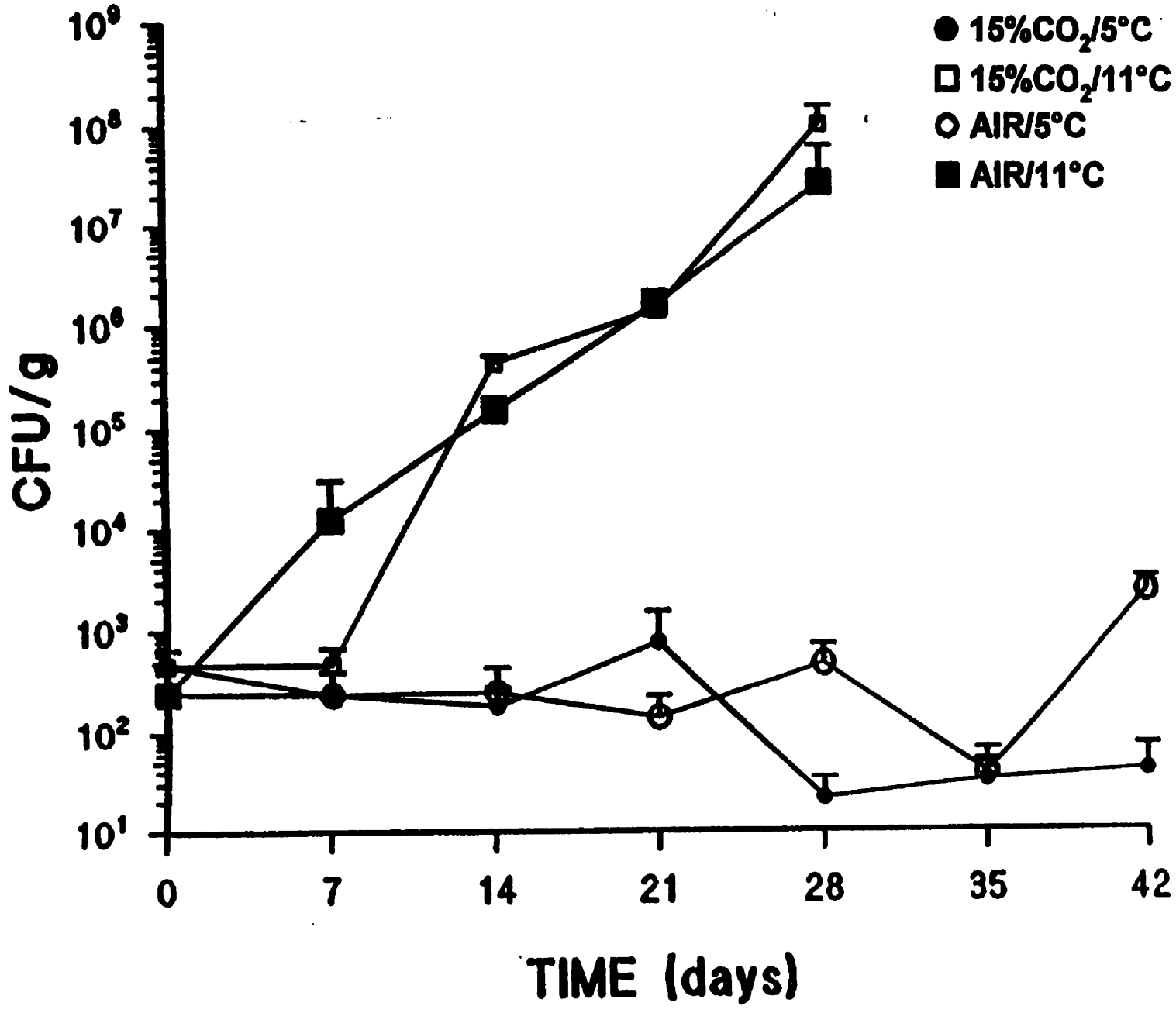

● 15%CO2/5°C
□ 15%CO2/11°C
○ AIR/5°C
■ AIR/11°C
CFU/g
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
0
7
14
21
28
35
42
TIME (days)

Figure 5. Survival and/or growth of *L. monocytogenes* in packaged beef (20) containing either 30%:70 % CO_2:N_2 or air. Bars represent the SD of the mean; n= 9. Appendix table: 3.

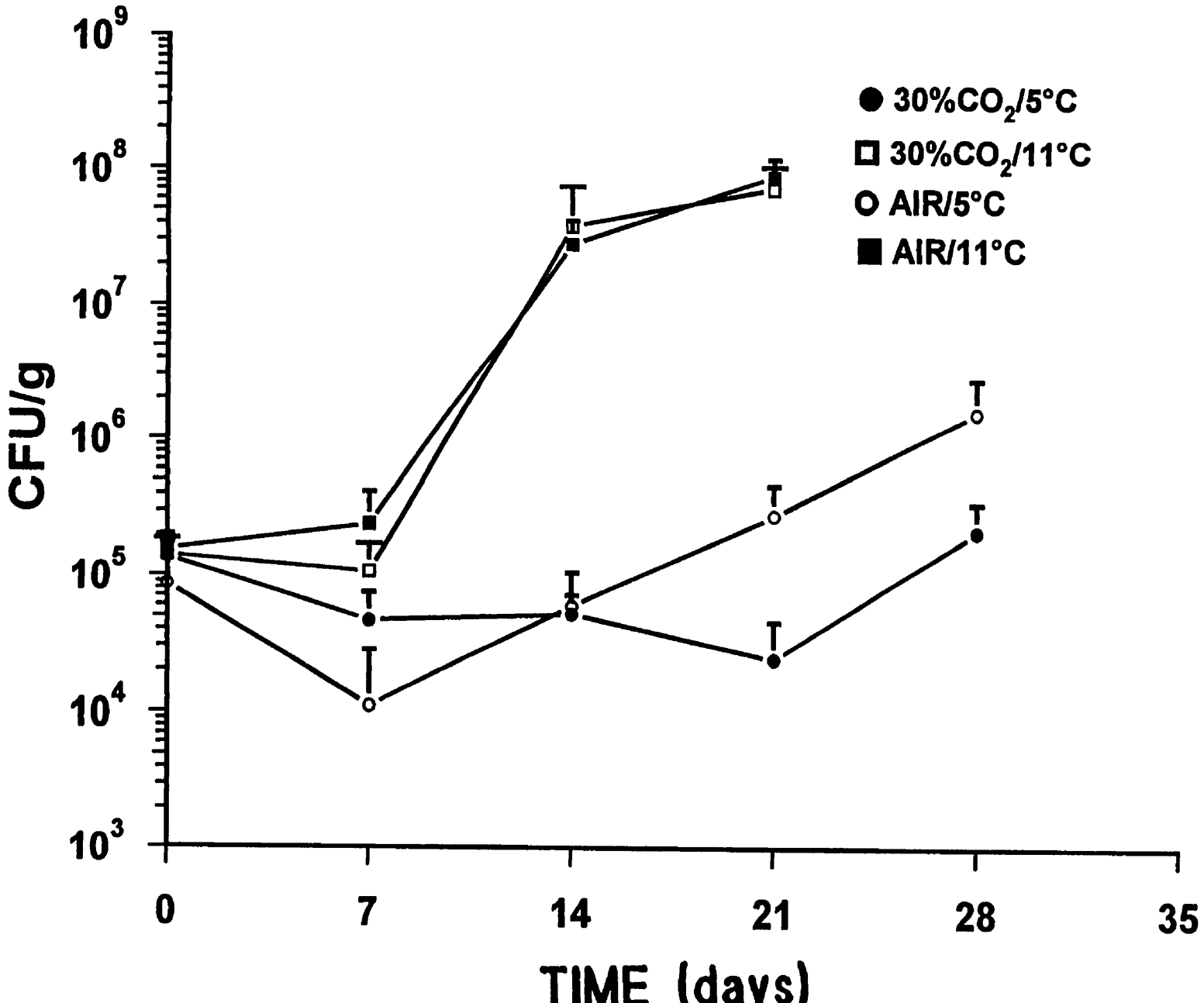
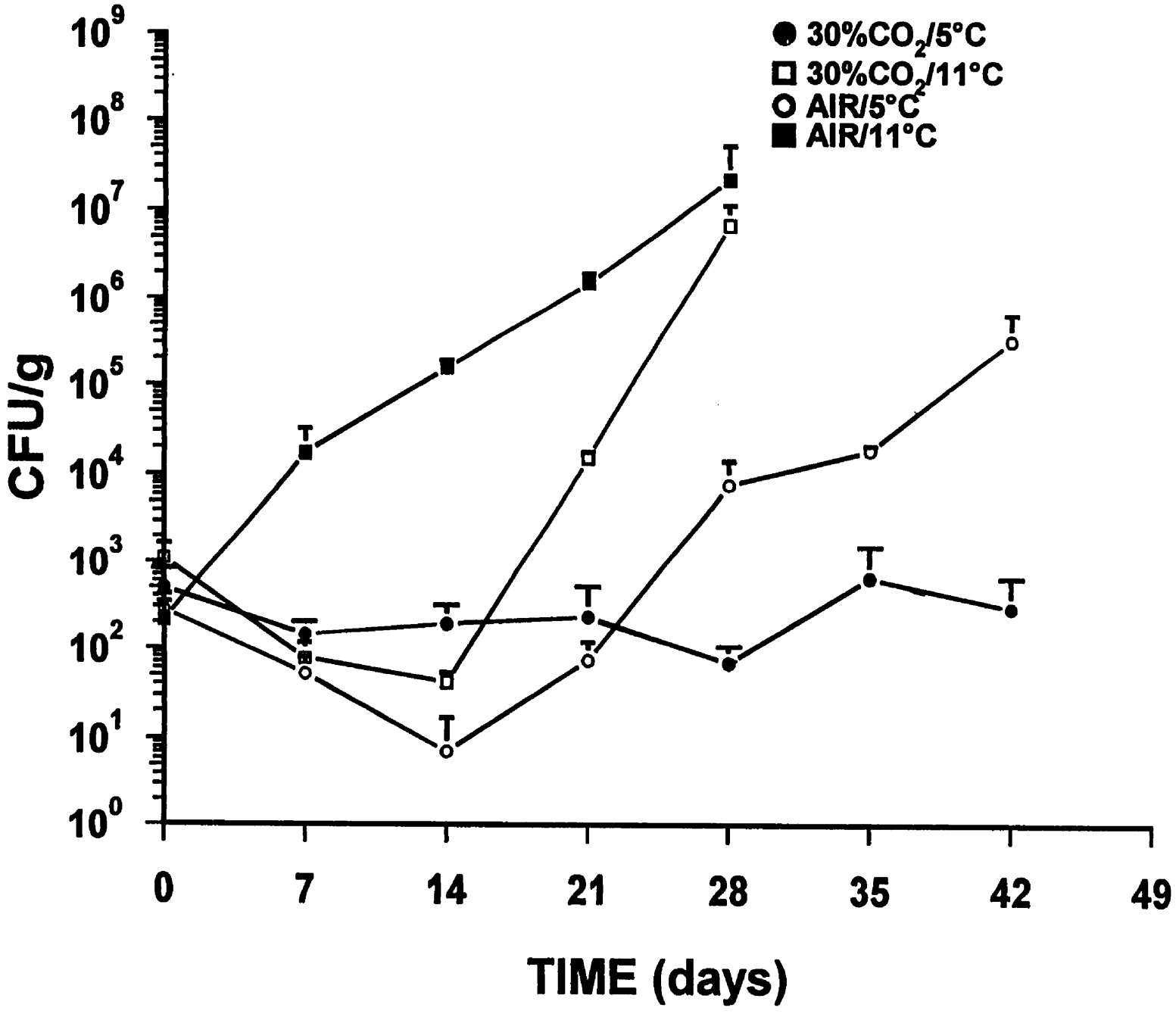

10^9
10^8
10^7
10^6
10^5
10^4
10^3
CFU/g
● 30%CO_2/5°C
□ 30%CO_2/11°C
○ AIR/5°C
■ AIR/11°C
0
7
14
21
28
35
TIME (days)

Figure 6. Survival and/or growth of *L. monocytogenes* in packaged beef (20) containing either 30%:70% CO_2:N_2 or air followed by irradiation at 1.75 kGy. Bars represent the SD of the mean; n= 9. Appendix table: 4.


30%CO2/5°C
30%CO2/11°C
AIR/5°C
AIR/11°C
CFU/g
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
7
14
21
28
35
42
49
TIME (days)

Survival and/ or Growth of *L. monocytogenes* in packaged beef (47)

The growth profile of *L. monocytogenes* in packaged beef containing 15:85% CO_2:N_2 and stored at 5^0C for 21 d appeared unaltered and is shown in Figure 7. The growth of listeriae in packaged beef with air appeared similar to that packaged in CO_2 during the initial 14 d storage period. Thereafter, however, the population increased from 10^5 CFU/g to 10^7 CFU/g. Storage at 11^0C resulted in a sharp increase in the population between 0 and 7 d (from 10^5 to 10^8 CFU/g) in both 15% CO_2 and air storage conditions. The presence of CO_2 at the elevated temperature appeared to have a minimal inhibitory effect when compared to the control at 5^0C.

After 7 d of storage at 5^0C, the listeriae population in beef (47) which was MAP and then irradiated, exhibited a gradual decrease in population from 10^2 to 10^1 CFU/g, as shown in Figure 8. A similar survival pattern was observed for listeriae in beef packaged with air. At 11^0C, the populations in both air and CO_2 increased with storage time. By 21 d, growth of listeriae reached 10^8 CFU/g for both 15% CO_2 and air packaged beef.

The growth and/ or survival of *L. monocytogenes* in packaged beef (47) containing 30:70% CO_2:N_2 and air is shown in Figure 9. At 5^0C with 30% CO_2, the population appeared to decrease initially from 10^5 to 10^4 CFU/g. In comparison, at 5^0C with air, the populations appeared to steadily increase; ca 0.5 log by 28 d. Storage at 11^0C resulted in a population increase to ca. 10^8 CFU by 21 d for both 30% CO_2 and air packaged beef.

Listeriae populations remained relatively constant in ground beef when stored at 5^0C following irradiation at 1.75 kGy (approximately 10^2 CFU/g) at least to 35 d as shown in Figure 10. Populations in air, however, increased to approximately 10^4 CFU/g at 42 d storage. Increasing the temperature to 11^0C resulted in an increase in the listeriae population at 7 d. In the case of beef stored with CO_2, the increase occurred at 14 d. Populations of 10^9 and 10^7 CFU/g were observed at 14 and 21 d, respectively.

Figure 7. Survival and/or growth of *L. monocytogenes* in packaged beef (47). containing either 15%:85% CO_2:N_2 or air. Bars represent the SD of the means; n = 9. Appendix table: 5

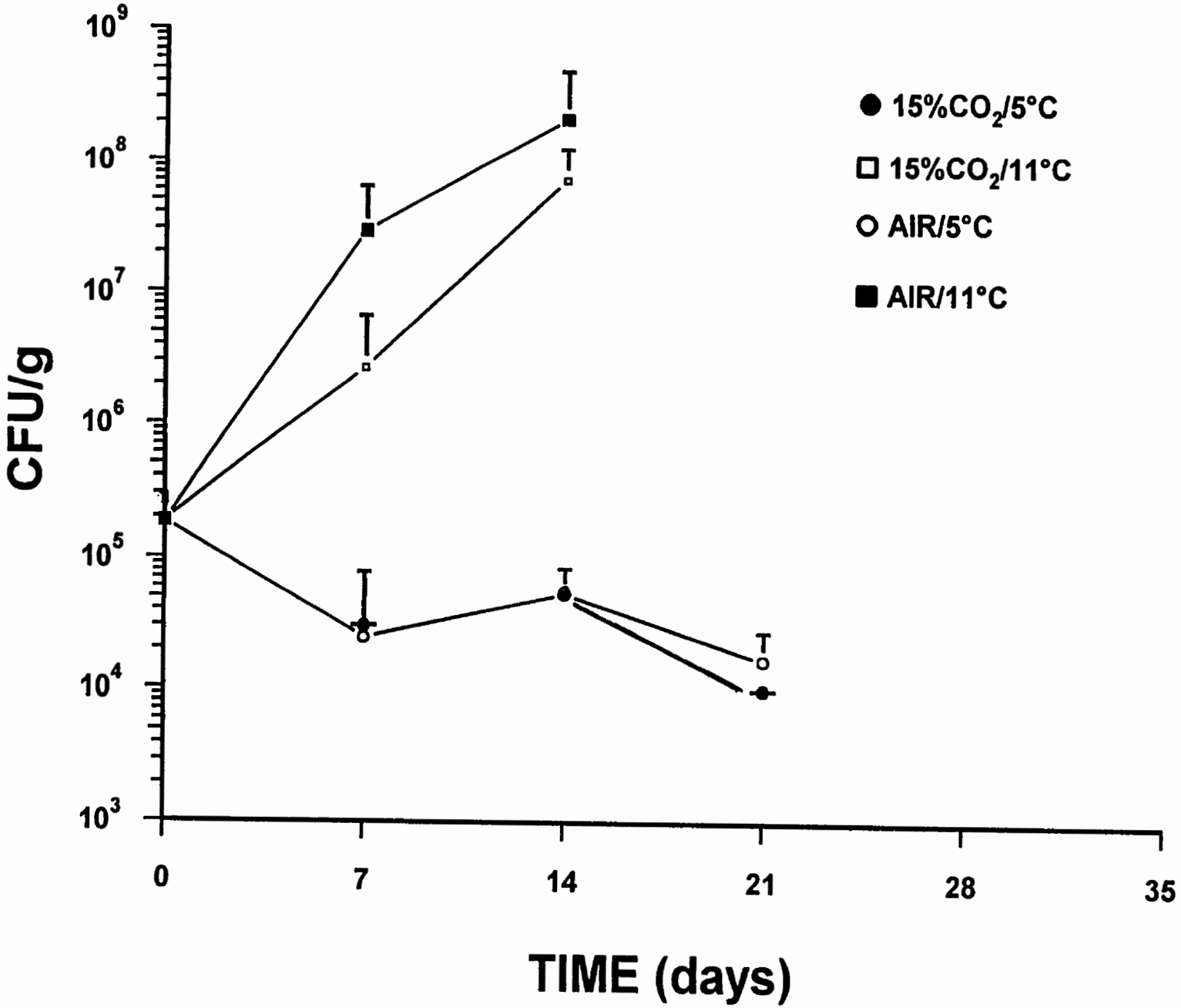

CFU/g
10^9
10^8
10^7
10^6
10^5
10^4
10^3
0
7
14
21
28
35
TIME (days)
15%CO2/5°C
15%CO2/11°C
AIR/5°C
AIR/11°C

Figure 8. Survival and/or growth of *L. monocytogenes* in packaged beef (47) containing either 15%:85% CO_2:N_2 or air followed by irradiation at 1.75 kGy. Bars represent the SD of the means; n = 9. Appendix table: 6.

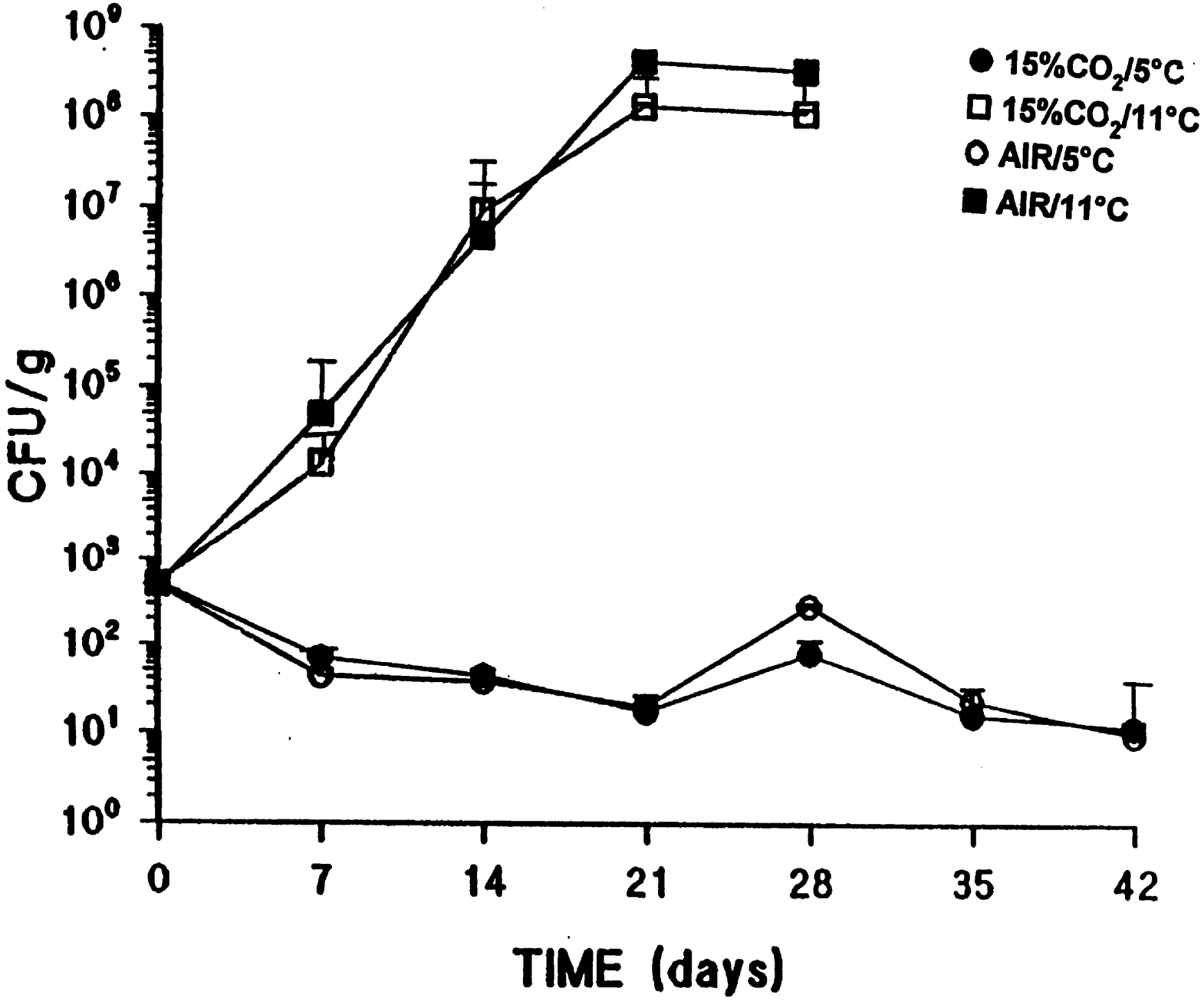

CFU/g
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
7
14
21
28
35
42
TIME (days)
● 15%CO_2/5°C
□ 15%CO_2/11°C
○ AIR/5°C
■ AIR/11°C

Figure 9. Survival and/or growth of *L. monocytogenes* in packaged beef (47) containing either 30%:70% CO_2:N_2 or air. Bars represent the SD of the means; n = 9. Appendix table: 7.

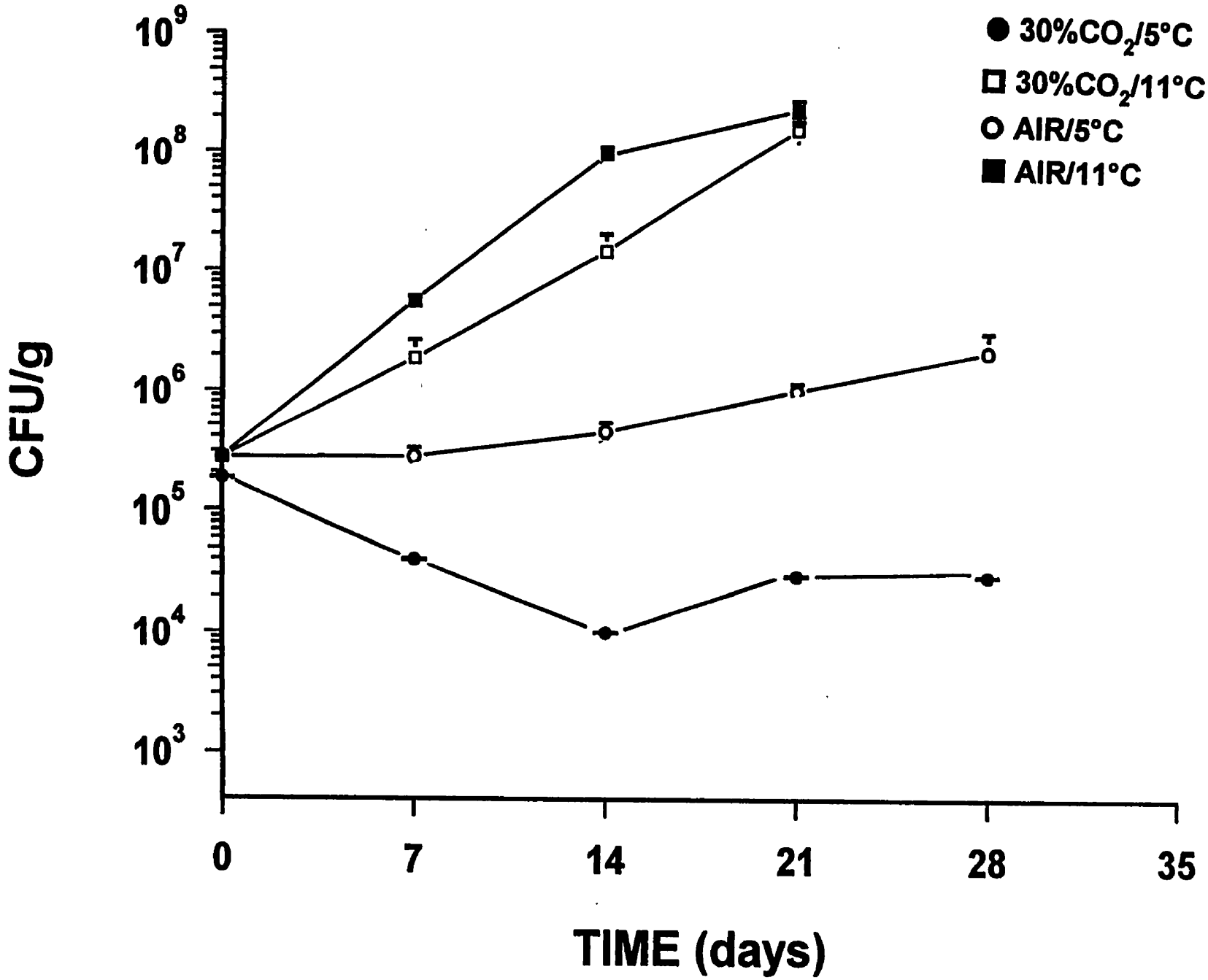
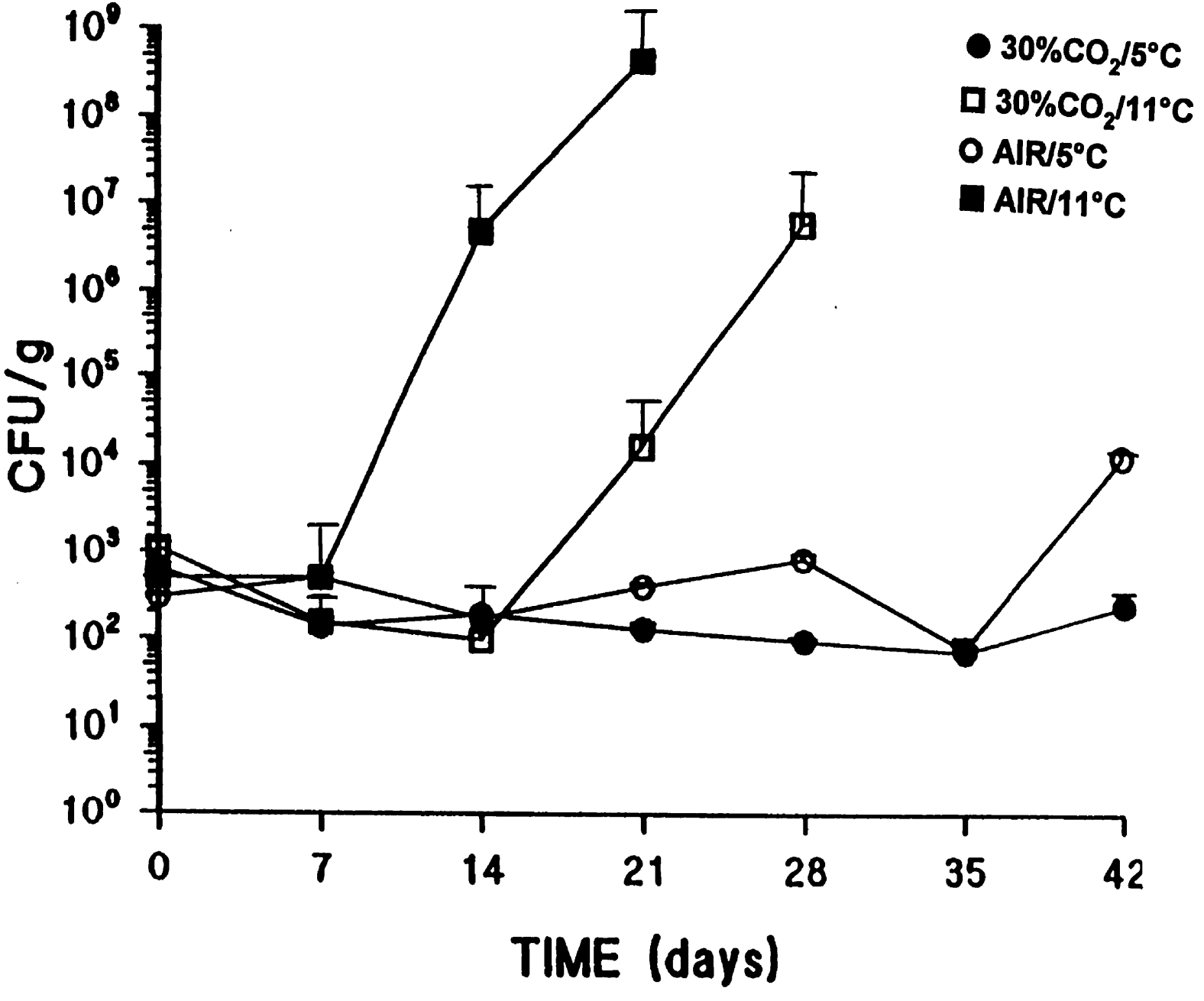

30%CO2/5°C
30%CO2/11°C
AIR/5°C
AIR/11°C
CFU/g
10^9
10^8
10^7
10^6
10^5
10^4
10^3
0
7
14
21
28
35
TIME (days)

Figure 10. Survival and/or growth of *L. monocytogenes* in packaged beef (47) containing either 30%:70% CO_2:N_2 or air followed by irradiation at 1.75 kGy. Bars represent the SD of the means; n = 9. Appendix table: 8.


30%CO2/5°C
30%CO2/11°C
AIR/5°C
AIR/11°C
CFU/g
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
7
14
21
28
35
42
TIME (days)

Survival and/or Growth of *L. monocytogenes* in beef (50)

The growth pattern of *L. monocytogenes* in 15:85% CO_2:N_2 at 5^0C over the storage period (21 d) appeared relatively constant at ca. 10^5 CFU/g, as shown in Figure 11. When packaged beef is exposed to air, the population also appeared relatively constant at ca. 10^5 CFU/g until 14 d. Thereafter, an increase to 10^7 CFU/g was observed by 21 d. Elevation of the storage temperature to 11^0C resulted in a sharp increase in the population to 10^8 CFU/g by 7 d. Thereafter the population appeared to have leveled off at 10^8 CFU/g in both the 15% CO_2 and air packaged beef.

The initial listeriae population (10^5 CFU/g) decreased to 10^2 CFU/g following irradiation (1.75 kGy) (Figure 12). Over the next 28 d of storage, the population in both 15% CO_2 and air remained relatively constant at 10^2 CFU/g.

L. monocytogenes maintained in 30:70% CO_2:N_2 at 5^0C decreased during storage from ca. 10^5 to10^4 CFU/g, as shown in Figure 13. In air, however, the survival pattern at the same temperature, appeared somewhat unchanged at ca. 10^5 CFU/g. At 11^0C, the listeriae population in beef packaged with either CO_2 or air increased immediately following radiation treatment. At 14 d of storage, 10^7 and 10^8 CFU/g were observed in CO_2 and air packaged beef, respectively.

Figure 11. Survival and/or Growth of *L. monocytogenes* in packaged beef (50) containing 15%:85% CO_2:N_2. Bars represent SD of the mean; n = 9. Appendix table: 9.

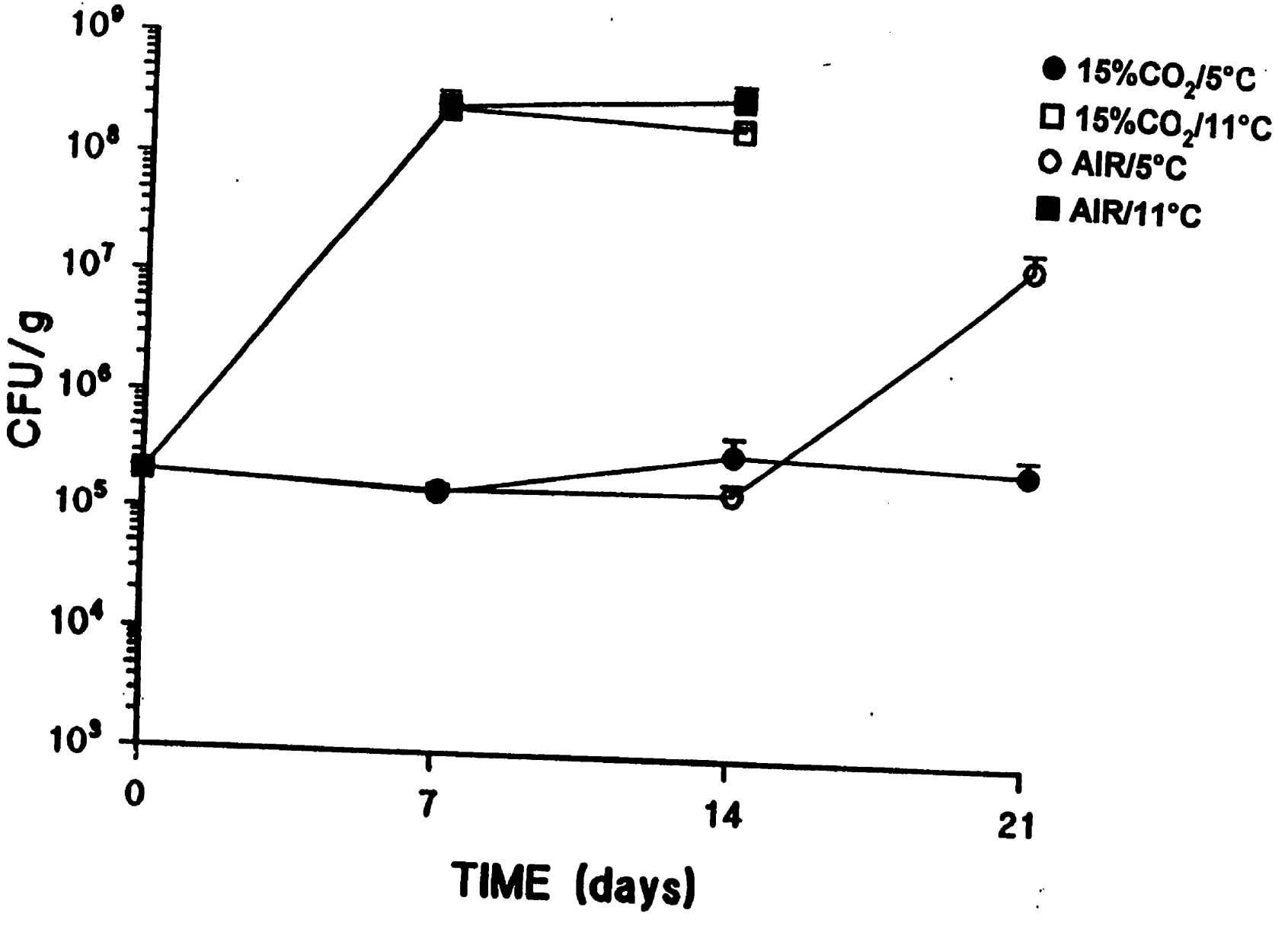

10^9
10^8
10^7
10^6
10^5
10^4
10^3
CFU/g
0
7
14
21
TIME (days)
15%CO_2/5°C
15%CO_2/11°C
AIR/5°C
AIR/11°C

Figure 12. Survival and/or growth of *L. monocytogenes* in packaged beef (50) containing either 15%:85% CO_2:N_2 or air followed by irradiation at 1.75 kGy. Bars represent SD of the mean; n = 9. Appendix table: 10.

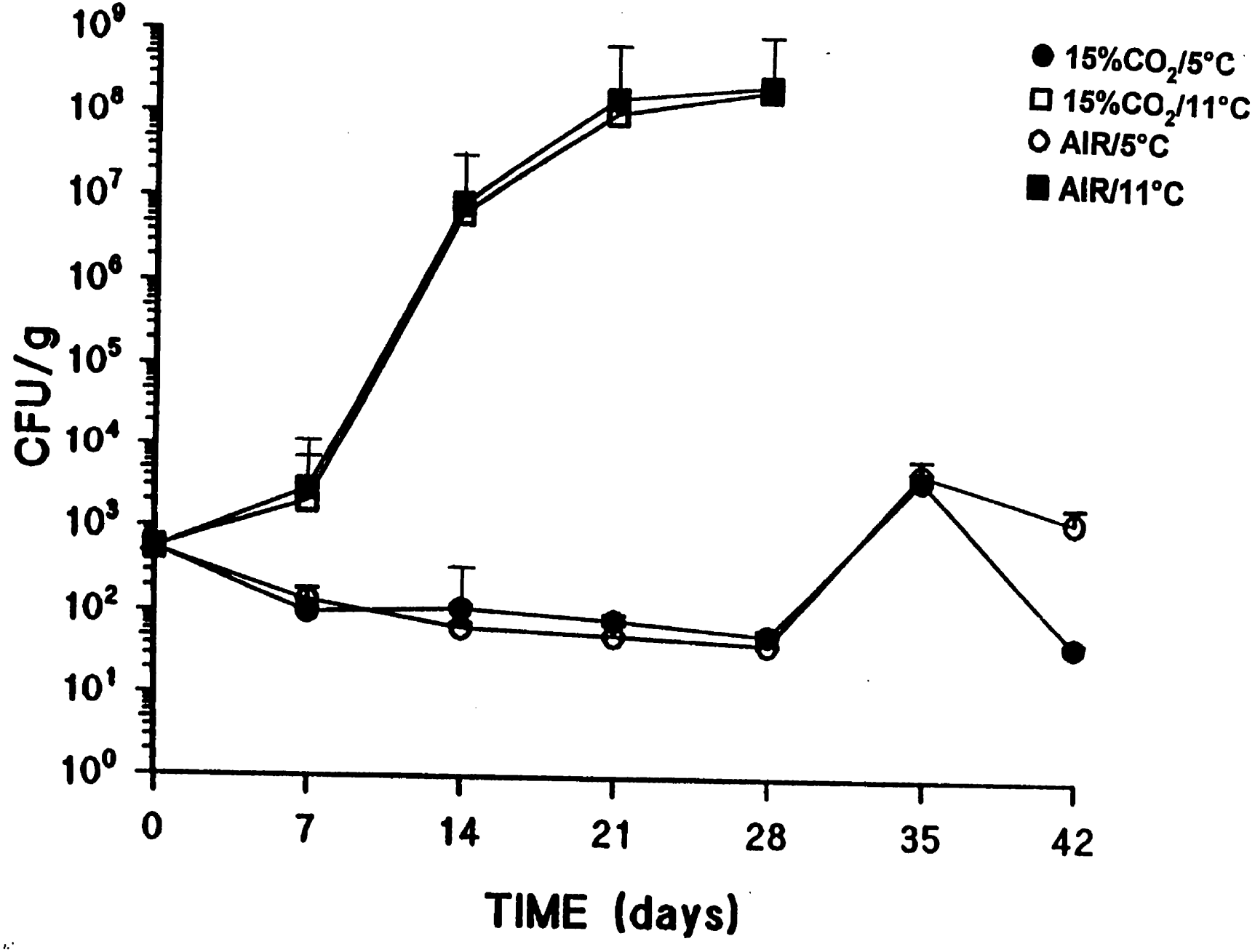

CFU/g
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
7
14
21
28
35
42
TIME (days)
15%CO2/5°C
15%CO2/11°C
AIR/5°C
AIR/11°C

Figure 13. Survival and/ or growth of *L. monocytogenes* in packaged beef (50) containing either 30%:70% CO_2:N_2 or air. Bars represent SD of the mean; n = 9. Appendix table: 11.

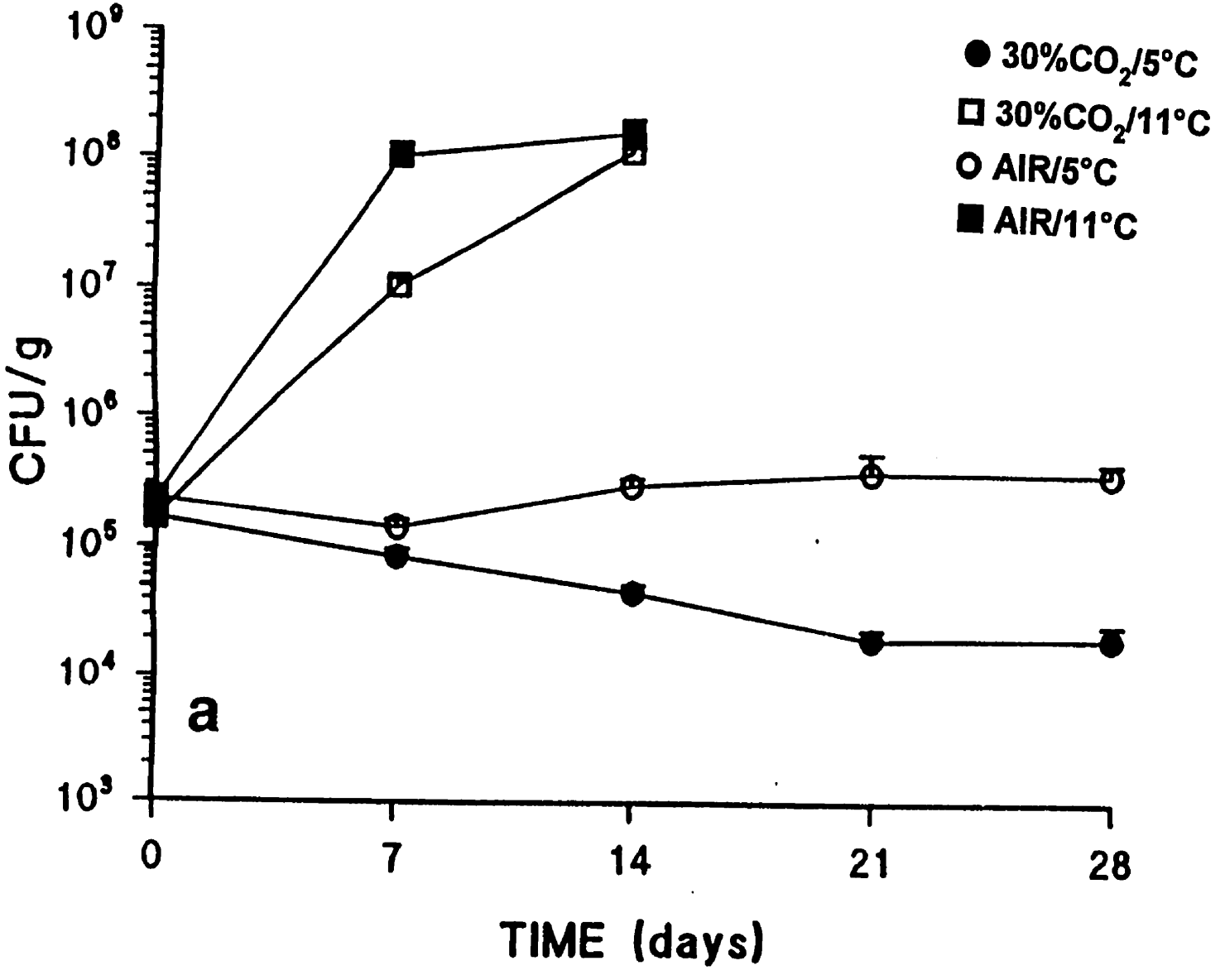
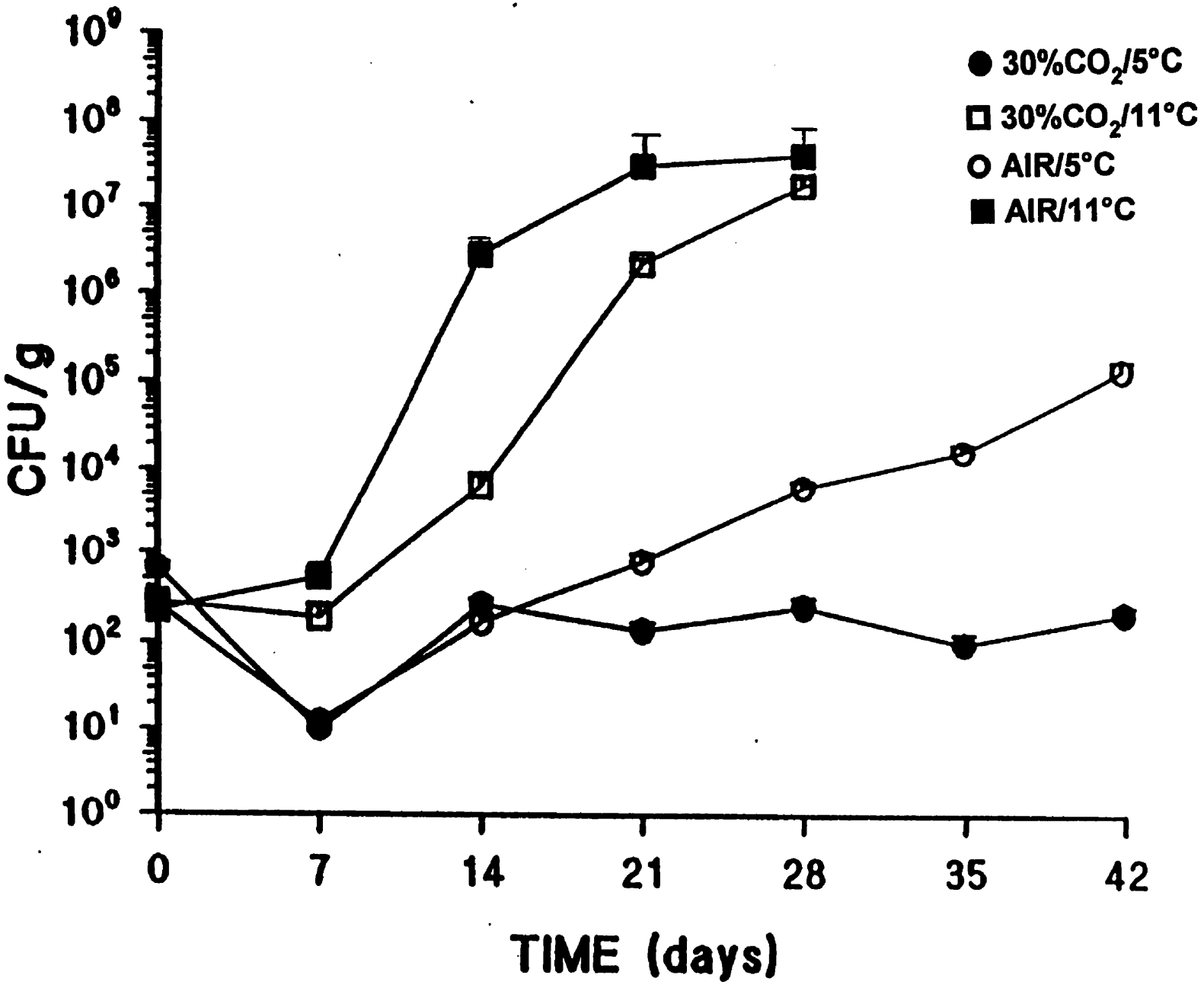

10⁹
10⁸
10⁷
10⁶
10⁵
10⁴
10³
CFU/g
a
0
7
14
21
28
TIME (days)
● 30%CO₂/5°C
□ 30%CO₂/11°C
○ AIR/5°C
■ AIR/11°C

Figure 14. Survival and growth of *L. monocytogenes* in packaged beef (50) containing either 30%:70% CO_2:N_2 or air followed by irradiation at 1.75 kGy. Bars represent the SD of the means; n = 9. Appendix table: 12.


CFU/g
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
7
14
21
28
35
42
TIME (days)
30%CO_2/5°C
30%CO_2/11°C
AIR/5°C
AIR/11°C

Following irradiation treatment (1.75 kGy), the initial population (10^5 CFU/g) decreased to ca. 10^2 CFU/g (Figure 14). At 5^0C in the presence of 30% CO_2, the listeriae population remained at 10^2 CFU/g throughout the entire 42 d storage period. However, in air at the same temperature, the population increased to approximately 10^6 CFU/g during the same time. When the storage temperature was increased to 11^0C, the population approached 10^7 CFU/g in both CO_2 and air packaged beef by day 28.

Survival and/or growth of *L. monocytogenes* in beef (54)

Populations of *L. monocytogenes* at 5^0C in either 15%:85% CO_2:N_2 or air appeared constant during storage at approximately 10^5 CFU/g, as shown in Figure 15. A gradual increase in growth, however, was observed when the storage temperature was increased to 11^0C. By day 14, ca. 10^8 and 10^7 CFU/g were obtained for beef stored in 15% CO_2 and air, respectively.

Initial populations decreased to 10^3 CFU/g following the application of 1.75 kGy. As shown in Figure 16, a slight decrease in population was observed when the beef was stored with 15% CO_2 and 5^0C. A similar trend was observed with beef stored in air. At 11^0C, the listeriae population in beef stored with either CO_2 or air increased. Maximal levels immediately were obtained at ca. 21 d (ca. 10^8 CFU/g).

The survival pattern of *L. monocytogenes* in either 30:85% CO_2:N_2 or air is presented in Figure 17. In the presence of CO_2 the population decreased during the first 7 d of storage by ca. 1 log. Thereafter, listeriae levels appeared constant. In

air, populations remained constant at ca. 10^5 CFU/g throughout storage. When the temperature was increased to 11^0C, listeriae in ground beef stored with CO_2, initially appeared to decrease (ca. 0.5 log). Thereafter, the population increased to ca. 10^7 by 14 d. In air, the listeriae population increased immediately at the outset of storage. The population at 14 d was ca. 0.5 log higher than that observed in CO_2 packaged beef.

The listeriae population decreased from 10^5 to 10^3 CFU/g following the application of 1.75 kGy as shown in Figure 18. At 5^0C, the listeriae population was observed to slowly decrease to ca. 10^2 CFU/g at 35 d when packaged in either 30% CO_2 or air. Elevation of the storage temperature to 11^0C resulted in an increase in the population reaching 10^8 CFU/g for both CO_2 and air by day 21.

Figure 15. Survival and/or growth of *L. monocytogenes* in packaged beef (54) containing either 15%:85% CO_2:N_2 or air. Bars represent the SD of the mean; n = 9. Appendix table: 13.

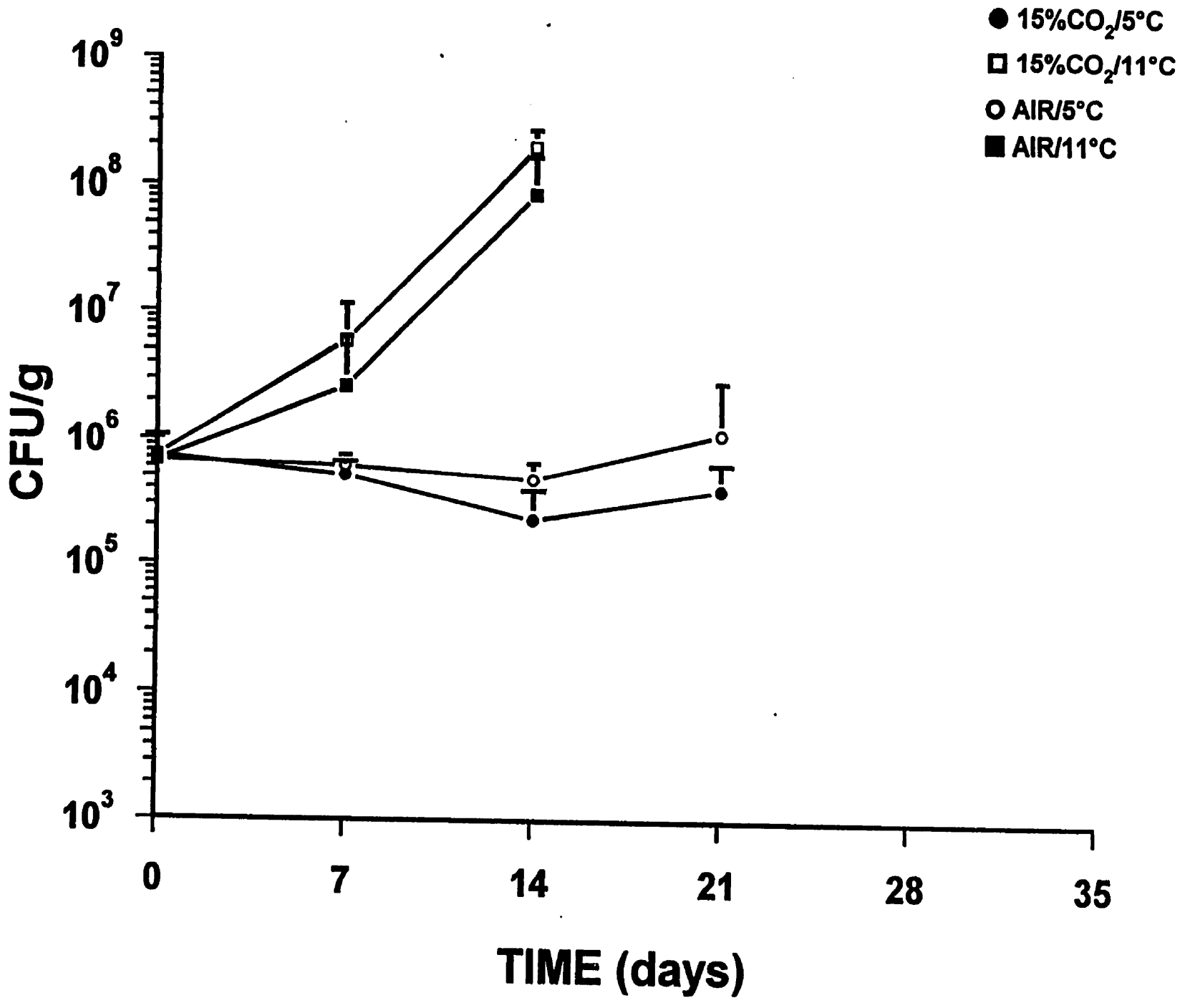

● 15%CO2/5°C
□ 15%CO2/11°C
○ AIR/5°C
■ AIR/11°C
CFU/g
10^9
10^8
10^7
10^6
10^5
10^4
10^3
0
7
14
21
28
35
TIME (days)

Figure 16. Survival and/or growth of *L. monocytogenes* in packaged beef (54) containing either 15%:85% CO_2:N_2 or air followed by irradiation at 1.75 kGy. Bars represent the SD of the mean; n = 9. Appendix table: 14.

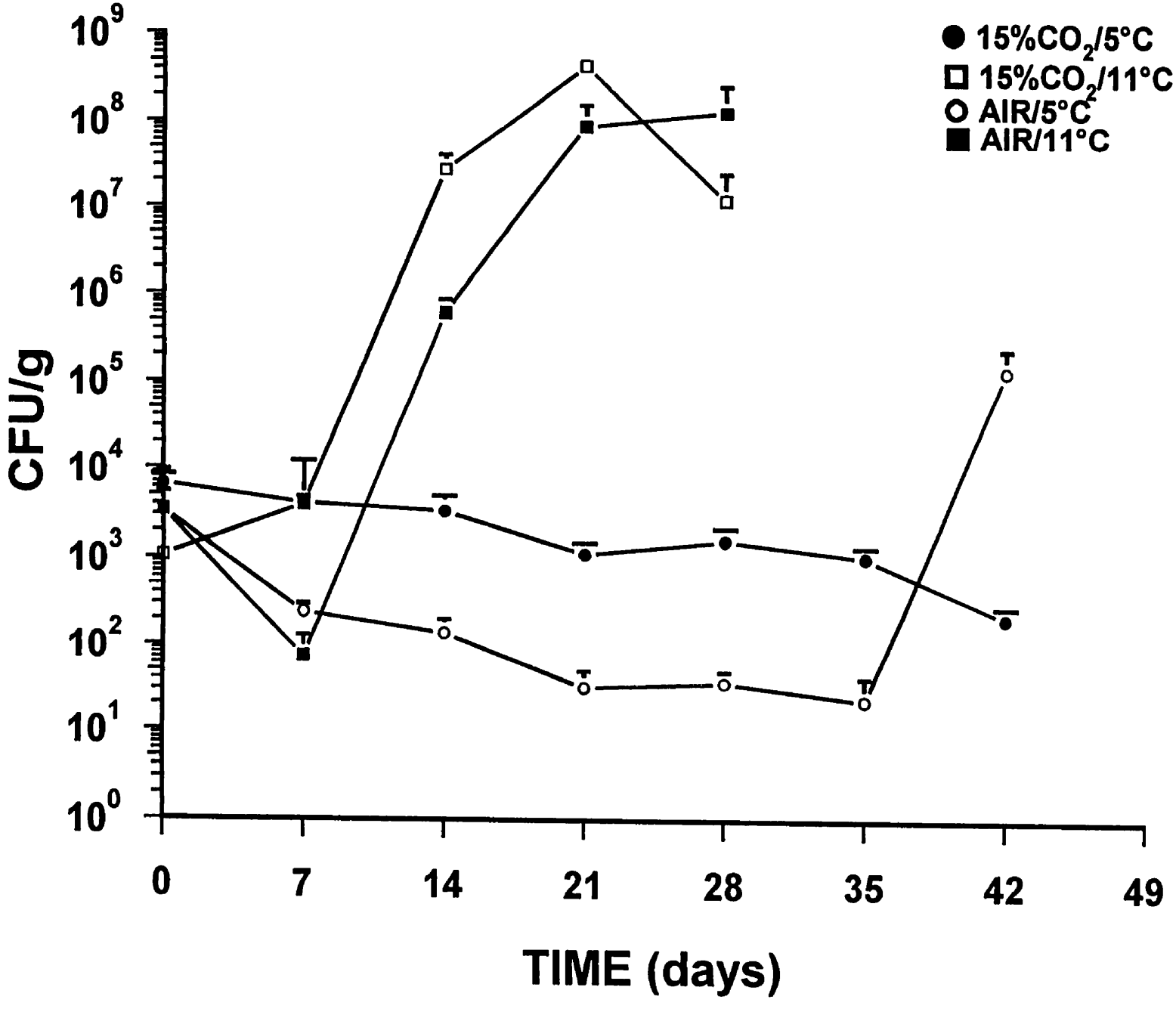

15%CO2/5°C
15%CO2/11°C
AIR/5°C
AIR/11°C
CFU/g
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
7
14
21
28
35
42
49
TIME (days)

Figure 17. Survival and/or growth of *L. monocytogenes* in packaged beef (54) containing either 30%:70% CO_2:N_2 or air. Bars represent the SD of the mean; n = 9. Appendix table: 15.

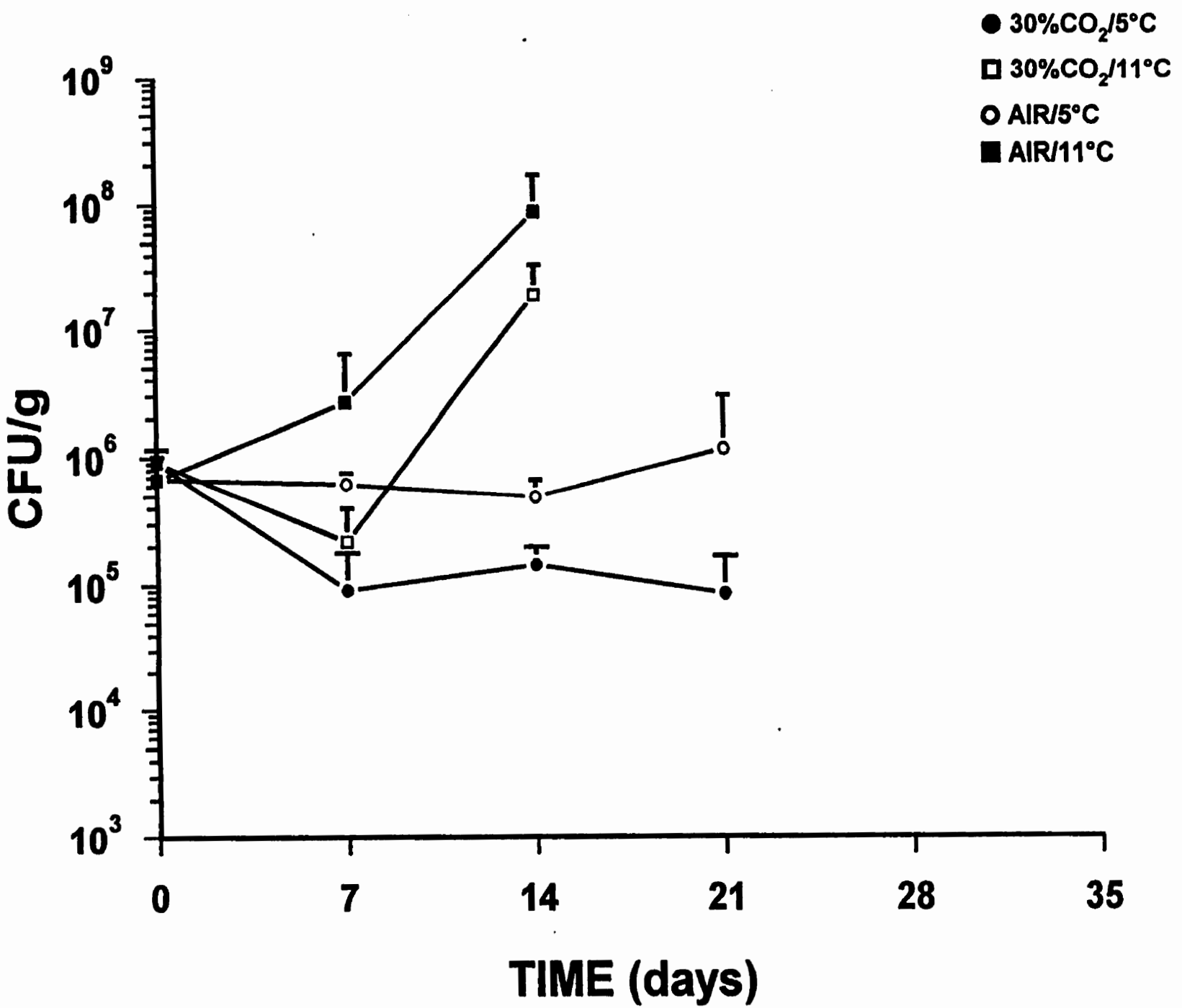
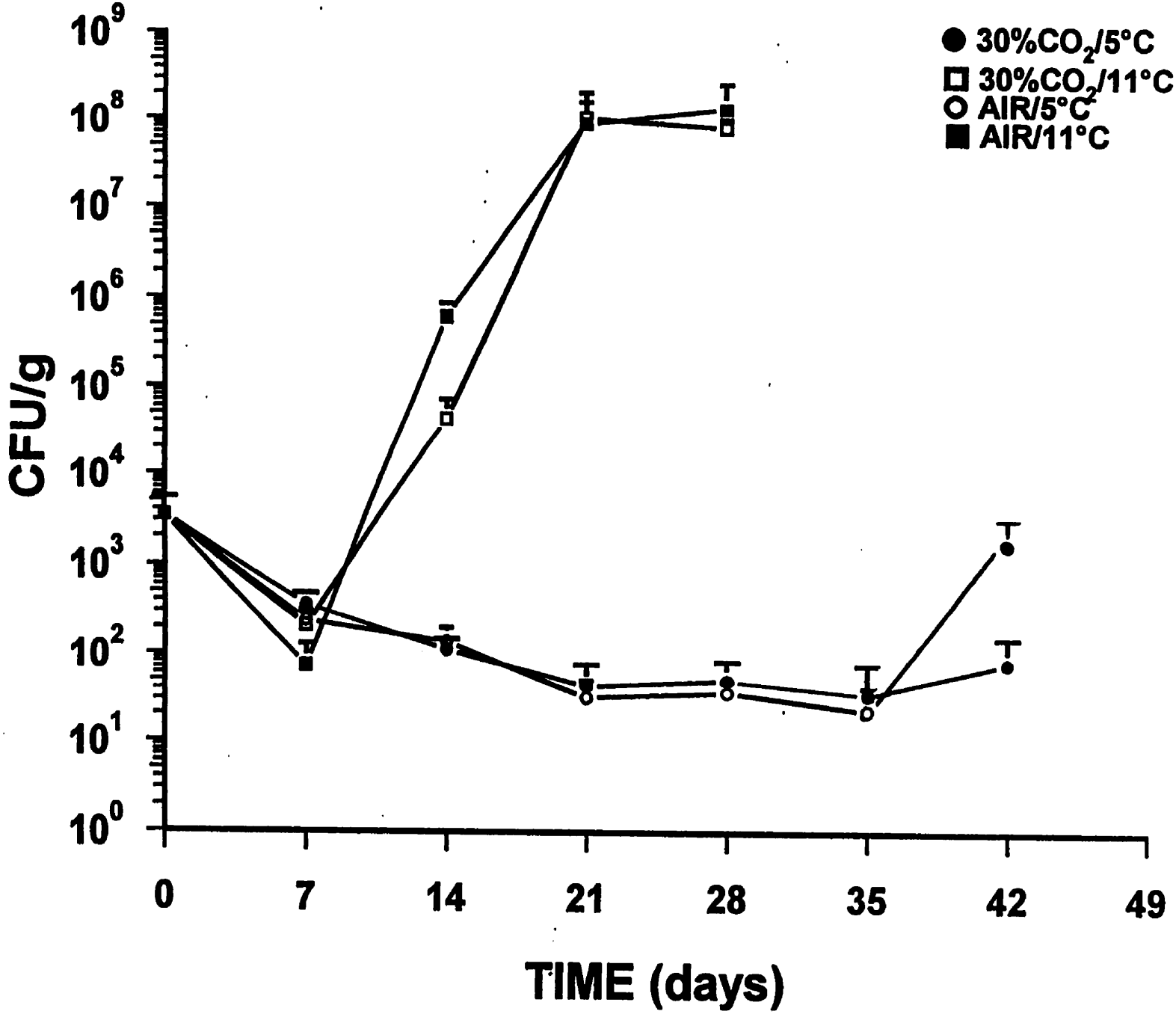

● 30%CO2/5°C
□ 30%CO2/11°C
○ AIR/5°C
■ AIR/11°C
CFU/g
10^9
10^8
10^7
10^6
10^5
10^4
10^3
0
7
14
21
28
35
TIME (days)

Figure 18. Survival and/or growth of *L. monocytogenes* in packaged beef (54) containing either 30%:70% CO_2:N_2 or air followed by irradiation at 1.75 kGy. Bars represent the SD of the mean; n = 9. Appendix table: 16.


30%CO2/5°C
30%CO2/11°C
AIR/5°C
AIR/11°C
CFU/g
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
7
14
21
28
35
42
49
TIME (days)

Qualitative assessment of pathogenicity

Listeria pathogenicity in terms of hemolysin activity was examined using extracts from inoculated beef (47) stored under various regimens. Direct identification of haemolysis on blood agar plates was confirmed by the presence of clear zones surrounding listeriae colonies (Table 3). There was no evidence of hemolysis resulting from the extracts obtained from the uninoculated meat.

Quantitative assessment of pathogenicity

Table 4 shows the death rate chick embryos following inoculation with a pure culture of *L. monocytogenes*. Death rates of 50% and 90% were observed 6 d after inoculation with 10^0 and 10^1 CFU/ ml, respectively. In contrast, inocula of 10^3 CFU/ ml or greater resulted in a 100% death rate, usually within day 4 of injection.

The death rate of chick embryos ranged from 80 to 100% (Table 5) when inoculated with extracts obtained from beef stored in air. In comparison, extracts obtained from been stored in CO_2 appeared to result in slightly higher mortality rates (90% to 100%). This effect was observed regardless of the storage temperature (Table 6 and 7).

The mortality rates of embryos following inoculation with extracts obtained from packaged and irradiated beef are shown in Tables 8, 9 and 10. Following irradiation, the undiluted extracts (10^1 CFU/ ml) resulted in mortality rates ranging from 80% to 90% and are similar to rates given by the control (Table 4). Undiluted extracts obtained from samples stored at 5^0C for 42 d, however, appeared to result in diminished mortality rates (50 % to 60 %). Changes in chick embryo mortality

were also observed with extracts obtained from samples stored at 11^{0}C especially in air (Table 8). In this respect, mortality rates decreased by 10 % and 40 % for the 10^{5} to 10^{4} and 10^{3} extract dilutions respectively, when compared to the controls (Table 4).

Table 3. Qualitative assessment of pathogenicity based on hemolysin activity from extracts of beef (47).

Storage Condition[1]	Temperature of Storage (^{0}C)	Haemolytic Activity[2]
Control[3]	–	Negative
Air	5	Positive
	11	positive
1.75 kGy + Air	5	positive
	11	positive
15% CO_2	5	positive
	11	positive
1.75 kGy + 15% CO_2	5	positive
	11	positive
30% CO_2	5	positive
	11	positive
1.75 kGy + 30% CO_2	5	positive
	11	positive

[1] Assessed after 7 d storage

[2] Positive hemolysis indicated by clear zones surrounding colonies on blood agar plates

[3] Control: extract from non-inoculated beef

Table 4. Death rate of embryos after inoculation with a pure culture of *L. monocytogenes*.

CFU/ ml[1]	Number of dead embryos on day						Total dead embryos[2]	Death rate (%)
	1	2	3	4	5	6		
10^0	0	1	2	1	1	0	5/10	50
10^1	1	1	4	1	0	1	9/10	90
10^3	2	3	4	1	--	--	10/10	100
10^4	5	5	--	--	--	--	10/10	100
10^5	4	5	1	--	--	--	10/10	100

[1]CFU/ml of beef (47) samples inoculated with *L. monocytogenes*

[2]Total no. of dead embryos/ total inoculated embryos

Table 5. Death rate of embryos after inoculation of *L. monocytogenes* from meat packaged in air.

Storage temp. (^{0}C)[1]	Storage Time (d)[2]	CFU/ ml[3]	Number of Dead Embryos on Day						Total dead embryos[4]	Death Rate (%)
			1	2	3	4	5	6		
	0	10^3	0	9	1	–	–	–	10/10	100
		10^4	2	8	–	–	–	–	10/10	100
		10^5	5	5	–	–	–	–	10/10	100
5	28	10^3	0	5	4	–	–	–	9/10	90
		10^4	0	6	4	–	–	–	10/10	100
		10^5	0	9	1	–	–	–	10/10	100
11	21	10^3	1	4	1	1	0	1	8/10	80
		10^4	5	4	0	0	0	0	9/10	90
		10^5	5	3	1	0	1	–	10/10	100

[1]Temperature of storage of beef (47) inoculated with *L. monocytogenes*
[2]Days of storage of beef (47) samples inoculated with *L. monocytogenes*
[3]CFU/ml of beef (47) samples inoculated with *L. monocytogenes*
[4]Total no. of dead embryos/ total inoculated embryos

Table 6. Death rate of embryos after inoculation with *L. monocytogenes* from meat packaged in 15% CO_2.

Storage temp. (^{0}C)[1]	Storage Time (d)[2]	CFU/ ml[3]	Number of Dead Embryos on Day						Total Dead embryos[4]	Death rate (%)
			1	2	3	4	5	6		
	Initial	10^3	7	2	0	0	1	–	10/10	100
		10^4	6	3	0	0	1	–	10/10	100
		10^5	8	2	–	–	–	–	10/10	100
5	21	10^3	4	5	–	–	–	–	9/10	90
		10^4	9	1	–	–	–	–	10/10	100
		10^5	7	3	–	–	–	–	10/10	100
11	14	10^3	3	6	0	0	0	0	9/10	90
		10^4	5	5	–	–	–	–	10/10	100
		10^5	6	3	0	0	0	0	9/10	90

[1]Temperature of storage of beef (47) inoculated with *L. monocytogenes*
[2]Days of storage of beef (47) samples inoculated with *L. monocytogenes*
[3]CFU/ml of beef (47) samples inoculated with *L. monocytogenes*
[4]Total no. of dead embryos/ total inoculated embryos

Table 7. Death rate of embryos after inoculation with *L. monocytogenes* from meat packaged in 30% CO_2.

Storage temp. (^{0}C)[1]	Storage time (d)[2]	CFU/ ml[3]	Number of Dead Embryos on Day						Total dead embryos[4]	Death rate (%)
			1	2	3	4	5	6		
	Initial	10^3	0	9	0	0	0	1	10/10	100
		10^4	1	9	–	–	–	–	10/10	100
		10^5	4	6	–	–	–	–	10/10	100
5	28	10^3	3	7	–	–	–	–	10/10	100
		10^4	2	8	–	–	–	–	10/10	100
		10^5	5	5	–	–	–	–	10/10	100
11	21	10^3	6	4	–	–	–	–	10/10	100
		10^4	9	1	–	–	–	–	10/10	100
		10^5	10	–	–	–	–	–	10/10	100

[1]Temperature of storage of beef (47) inoculated with *L. monocytogenes*
[2]Days of storage of beef (47) samples inoculated with *L. monocytogenes*
[3]CFU/ml of beef (47) samples inoculated with *L. monocytogenes*
[4]Total no. of dead embryos/ total inoculated embryos

Table 8. Death rate of embryos after inoculation with *L. monocytogenes* from meat packaged in air following irradiation with 1.75kGy.

Storage temp. (^{0}C)[1]	Storage time (d)[2]	CFU/ ml[3]	Number of Dead Embryos on Day						Total dead embryos[4]	Death rate (%)
			1	2	3	4	5	6		
	Initial	10^0	0	1	2	1	1	0	5/10	50
		10^1	1	1	5	1	0	0	8/10	80
5	42	10^0	0	2	2	0	0	0	4/10	40
		10^1	0	0	2	1	2	0	5/10	50
11	28	10^3	0	3	2	0	0	1	6/10	60
		10^4	1	7	1	0	0	0	9/10	90
		10^5	1	8	0	0	0	0	9/10	90

[1]Temperature of storage of beef (47) inoculated with *L. monocytogenes*
[2]Days of storage of beef (47) samples inoculated with *L. monocytogenes*
[3]CFU/ml of beef (47) samples inoculated with *L. monocytogenes*
[4]Total no. of dead embryos/ total inoculated embryos

Table 9. Death rate of embryos after inoculation with *L. monocytogenes* from meat packaged 15% CO_2 following irradiation of 1.75 kGy.

Storage temp. (^{0}C)[1]	Storage Time (d)[2]	CFU/ ml[3]	Number of Dead Embryos on Day						Total dead embryos[4]	Death rate (%)
			1	2	3	4	5	6		
	Initial	10^0	1	0	3	0	0	0	4/10	40
		10^1	2	4	1	1	0	0	8/10	80
5	42	10^0	1	0	3	0	1	0	5/10	50
		10^1	2	2	0	0	2	0	6/10	60
11	28	10^3	0	6	4	0	0	0	10/10	100
		10^4	5	4	1	0	0	0	10/10	100
		10^5	2	7	1	0	0	0	10/10	100

[1]Temperature of storage of beef (47) inoculated with *L. monocytogenes*
[2]Days of storage of beef (47) samples inoculated with *L. monocytogenes*
[3]CFU/ml of beef (47) samples inoculated with *L. monocytogenes*
[4]Total no. of dead embryos/ total inoculated embryos

Table 10. Death rate of embryos after inoculation with *L. monocytogenes* from meat packaged in 30% CO_2 following irradiation with 1.75kGy.

Storage temp. (^{0}C)[1]	Storage time (d)[2]	CFU/ ml[3]	Number of Dead Embryos on Day						Total dead embryos[4]	Death rate (%)
			1	2	3	4	5	6		
	Initial	10^0	0	2	1	0	0	0	3/10	30
		10^1	0	9	0	0	0	0	9/10	90
5	42d	10^0	0	1	5	0	0	0	6/10	60
		10^1	0	3	0	2	0	0	5/10	50
11	28d	10^3	1	5	3	1	0	0	10/10	100
		10^4	2	5	0	1	2	--	10/10	100
		10^5	4	4	1	0	1	0	10/10	100

[1]Temperature of storage of beef (47) inoculated with *L. monocytogenes*
[2]Days of storage of beef (47) samples inoculated with *L. monocytogenes*
[3]CFU/ml of beef (47) samples inoculated with *L. monocytogenes*
[4]Total no. of dead embryos/ total inoculated embryos

Mathematical modeling

A quantitative model can be developed describing the effect of temperature and atmospheric conditions for the growth of *L. monocytogenes* growing in different levels of protein. Assuming that the quantitative growth of the organism (dS/dt) is proportional to the quantity of the organism and it depends on the proportionality constant μ, it can be stated mathematically:

$$\frac{dS}{dt} = \mu S \qquad (7)$$

The proportionality constant μ, which is also known as the specific growth rate can decay with time due to the degradation of the development of *L. monocytogenes* usually, this is caused by overpopulation.

$$\frac{d\mu}{dt} = -D\mu \qquad (8)$$

where D is the decay in the specific growth rate of the organism.

After integrating Equation 8, it becomes:

$$\int_{\mu_0}^{\mu} \frac{d\mu}{\mu_0} = -D\int_{0}^{t} dt \qquad (9)$$

where, t is the growth time to the degradation of development of the organism.

The solution to the above equation is as follows:

$$\ln \mu - \ln \mu_0 = \frac{-D}{(t - t_0)} \qquad (10)$$

where, t_o is at time of inoculation of the organism to the medium, in this case ground beef and μ_o is the value of μ at $t = 0$.

These parameters are known in mathematics as initial conditions. By introducing them to the above equation, we obtain:

$$\ln \frac{\mu}{\mu_o} = -Dt \qquad (11)$$

Taking the exponents of both sides

$$\frac{\mu}{\mu_o} = \exp(-Dt) \qquad (12)$$

and rearranging we obtain:

$$\mu = \mu_o \exp(-Dt) \qquad (13)$$

By substituting Equation 13 into Equation 7, the quantitative growth of *L. monocytogenes* can be expressed as:

$$\frac{dS}{dt} = (\mu_o \exp(-Dt))\, S \qquad (14)$$

By sorting the variables S and dt, Equation 14 becomes:

$$\frac{dS}{dt} = (\mu_o \exp(-Dt))\, dt \qquad (15)$$

and

$$\frac{dS}{dt} = \mu_o \ (\exp(-Dt))\, dt \qquad (16)$$

By superimposing the integration limits:

$$\int_{So}^{S} \frac{dS}{dt} = \mu_o \int_{to}^{t} (\exp(-Dt))\, dt \qquad (17)$$

Integration of the above equation gives:

$$\ln S - \ln S_o = \frac{\mu_o}{D} (\exp(-Dt) - \exp(-Dt_o)) \qquad (18)$$

for $t_o = 0$ and the exponential expression becomes one ($\exp(0) = 1$). Thus, the equation becomes:

$$\ln \frac{S}{S_o} = \frac{-\mu_o}{D} (\exp(-Dt) - 1) \qquad (19)$$

This can also be written as:

$$\ln \frac{S}{S_o} = \frac{\mu_o}{D} (1 - \exp(-Dt)) \qquad (20)$$

The fraction, $\frac{\mu_o}{D}$ can be expressed as μ_o^*. Therefore, the equation becomes $\ln \frac{S}{S_o} = \mu_o^* (1 - \exp(-Dt))$. Finally the quantitative growth of *L. monocytogenes*, or the growth ratio can be expressed as:

$$\frac{S}{S_o} = \exp(\mu_o^* (1 - \exp(-Dt)) \qquad (21)$$

Application of the growth ratio

The growth ratio, $\frac{S}{S_o}$, can be applied to the growth of *L. monocytogenes* in beef (20), beef (47), beef (50) and beef (54) ground beef stored between 5^0C and 11^0C. This ratio demonstrates the effect of the atmosphere on the growth of the organism. By plotting time against the growth ratio, the impact of these factors can be seen as in Figures 19 to 26. These figures show the data collected in the form of symbols for three different storage conditions and meat samples with various

protein contents. The lines in the figures indicate the best fit curves obtained using the developed mathematical model in the form of equation $\frac{S}{S_o} = \exp(\mu_o^* (1 - \exp(-Dt))$.

Based on the obtained results and the derived Equation 21 the proportionality constant, μ_0, shows decay in the specific growth of the organism for particular conditions (for example, CO_2 and fat content). The ratio, $\frac{S}{S_o}$, was presented as a function of time for beef of various protein content and subjected to various CO_2 concentrations and storage temperatures. The ratio, $\frac{S}{S_o}$, was described by Equation 21. The best fit line generated was found using TK Solver Program. Since Equation 21 has two unknowns, this equation was applied two times so that the number of equations used equals to the same amount of unknowns. By applying the experimental results, TK Solver Program generated the values for μ_0^* and D. These generated results are presented in Table 11 for 5^0C and 11^0C.

The goodness of fit can be checked in Figures 19-22 for 5^0C and Figures 23-26 for 11^0C. The data points indicate the averages of up to nine values. The solid lines in the figures indicated the fitness of Equation 21. In the case when the experimental data points are presented as $\frac{S}{S_o}$, this ratio decrease with time. At t=0, the simulation line at best could only follow the horizontal line (for example, Figure 22 represents this situation for 15% and 30% CO_2) or a positively inclining horizontal line.

Equation 21 represents the growth phase or the stationary phase of the organism; it cannot represent the death phase. Thus, when data points $\frac{S}{S_o}$ become less than 1, the values of the simulation equation are also less than 1. An example of this are the growth curves at 15% and 30% CO2 in Figure 22. Note that the increments of the y-axis in Figure 22 has been expanded for a better visual presentation and for discussion purposes.

Figure 19. Growth ratio as a function of time for beef (20) stored at 5^{0}C.

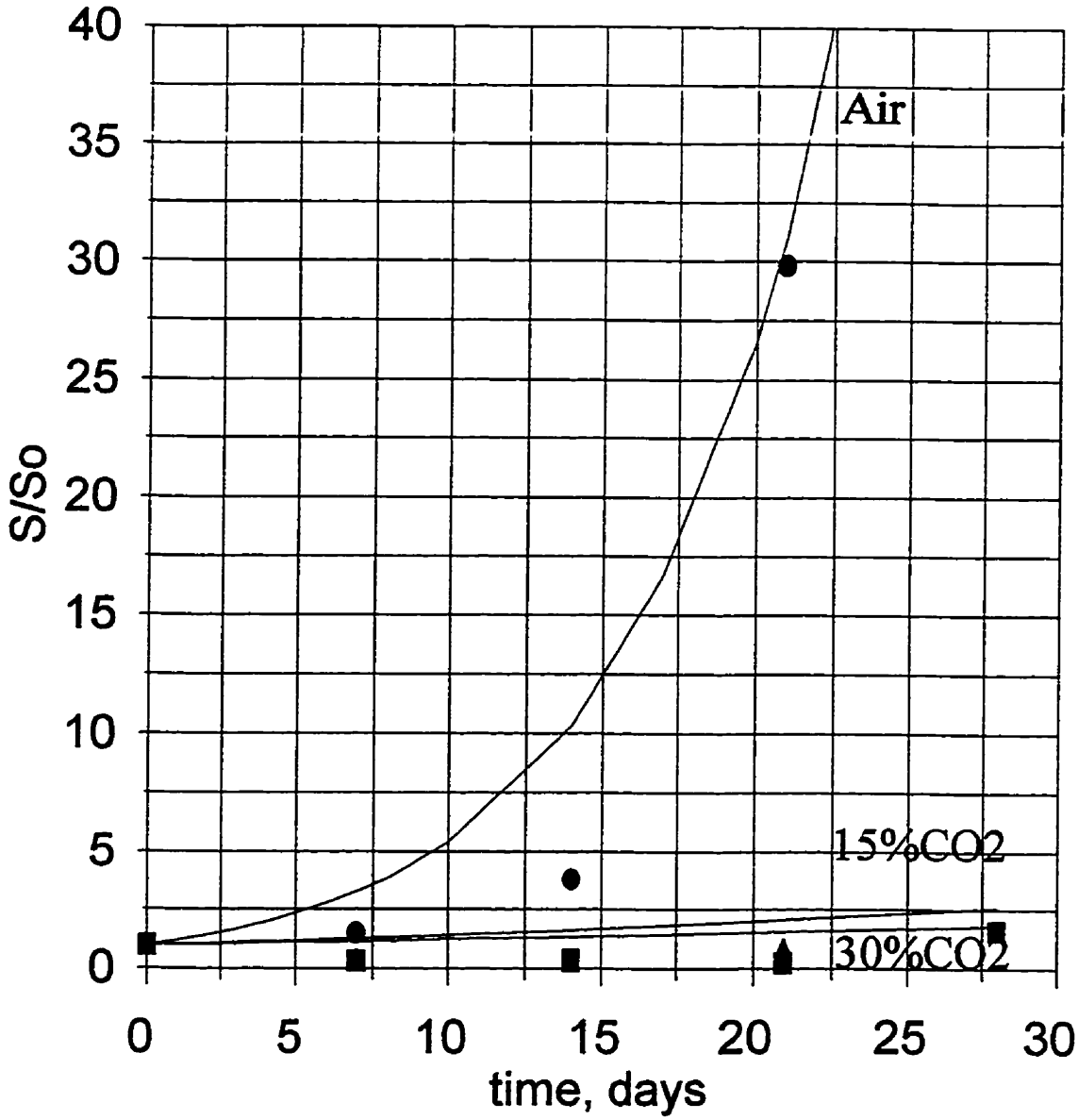

40
35
30
25
20
15
10
5
0
S/So
Air
15%CO2
30%CO2
0
5
10
15
20
25
30
time, days

Figure 20. Growth ratio as a function of time for beef (47) stored at 5^0C

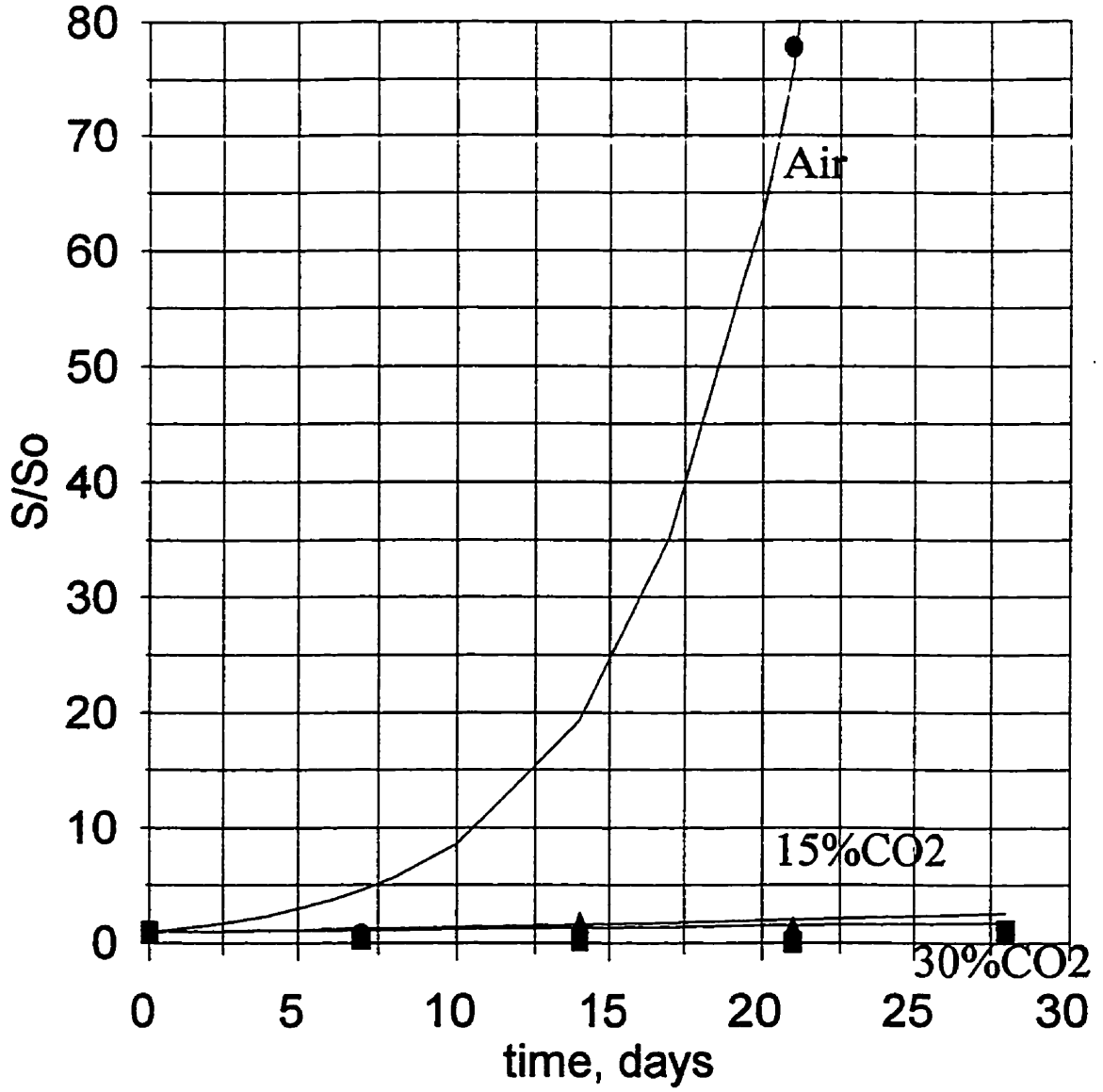

80
70
60
50
40
30
20
10
0
S/S0
Air
15%CO2
30%CO2
0
5
10
15
20
25
30
time, days

Figure 21. Growth ratio as a function of time for beef (50) stored at 5^0C.

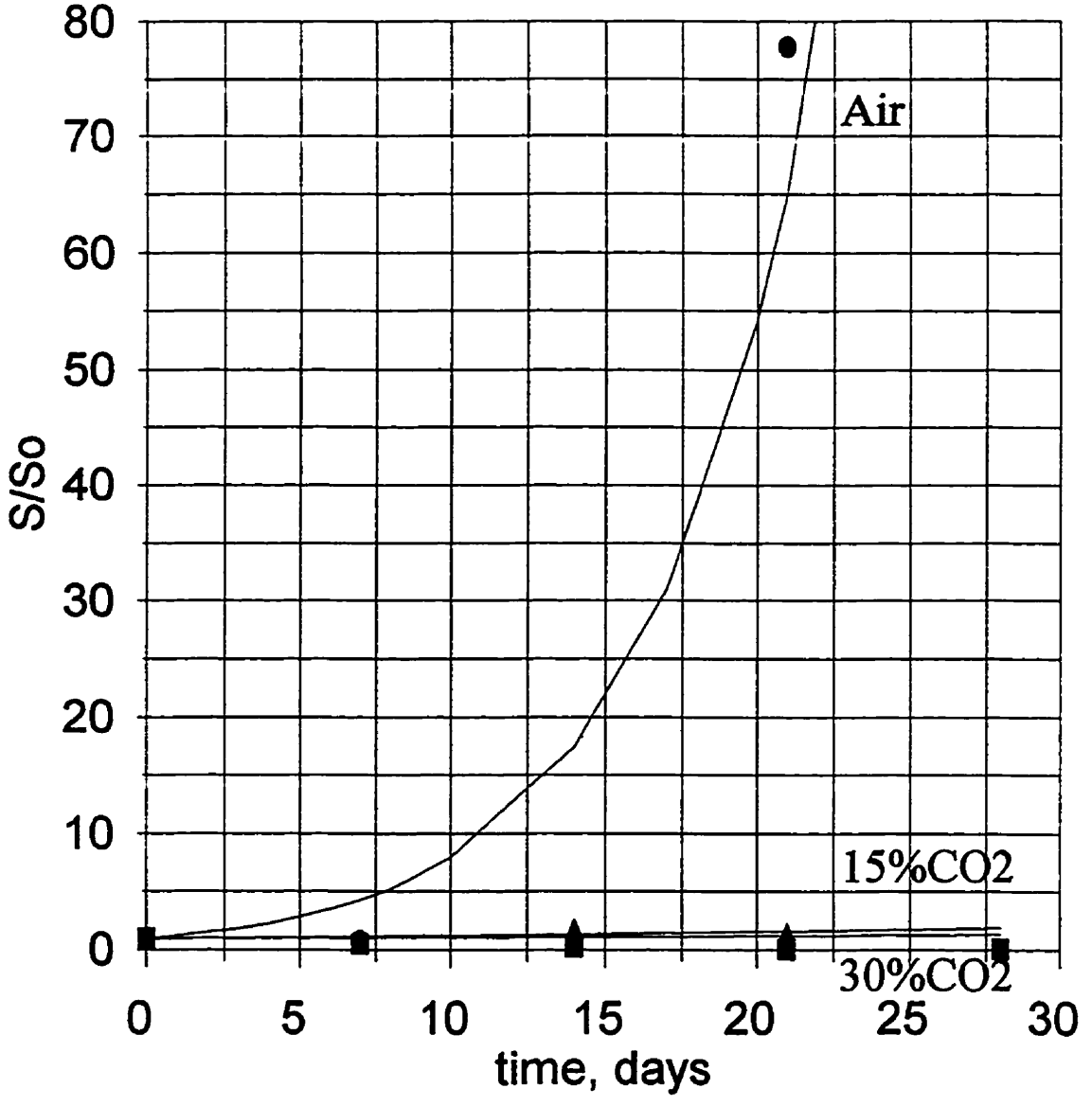

80
70
60
50
40
30
20
10
0
S/So
Air
15%CO2
30%CO2
0
5
10
15
20
25
30
time, days

Figure 22. Growth ratio as a function of time for beef (54) stored at 5^0C.

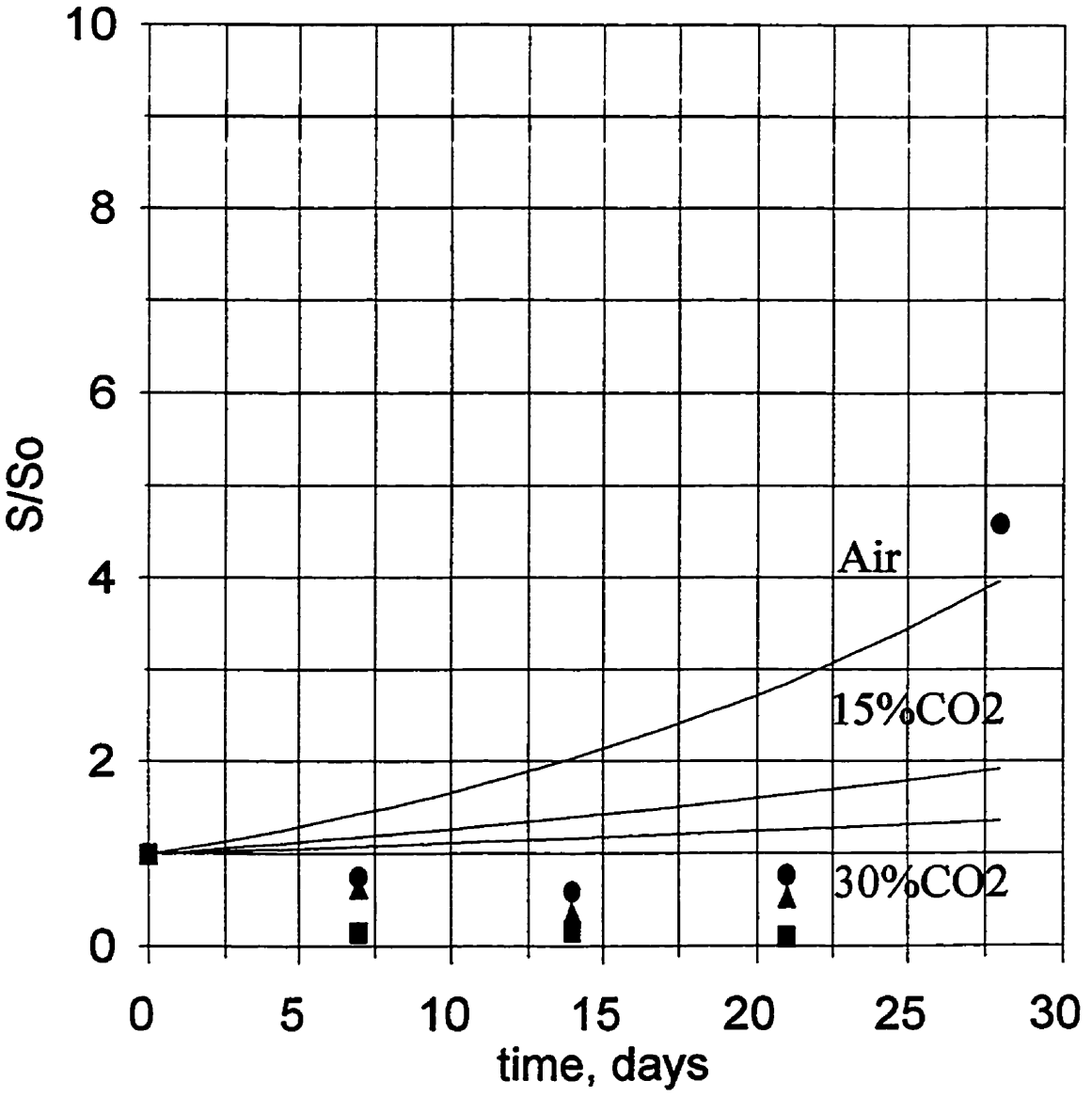

10
8
6
4
2
0
S/S0
Air
15%CO2
30%CO2
0
5
10
15
20
25
30
time, days

Figure 23. Growth ratio as a function of time for beef (20) stored at 11^{0}C.

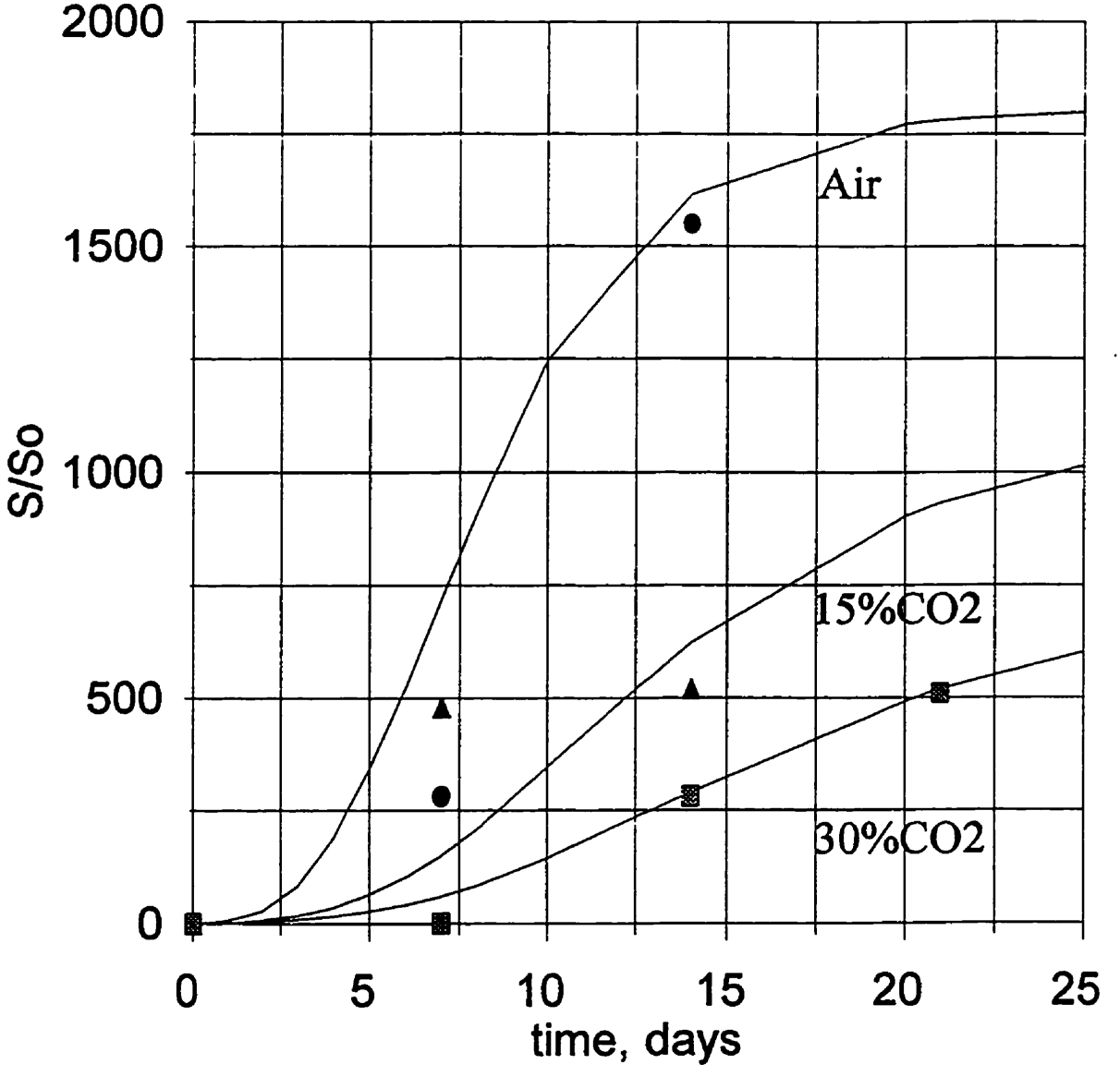

2000
1500
1000
500
0
S/S0
Air
15%CO2
30%CO2
0
5
10
15
20
25
time, days

Figure 24. Growth ratio as a function of time for beef (47) stored at 11^{0}C.

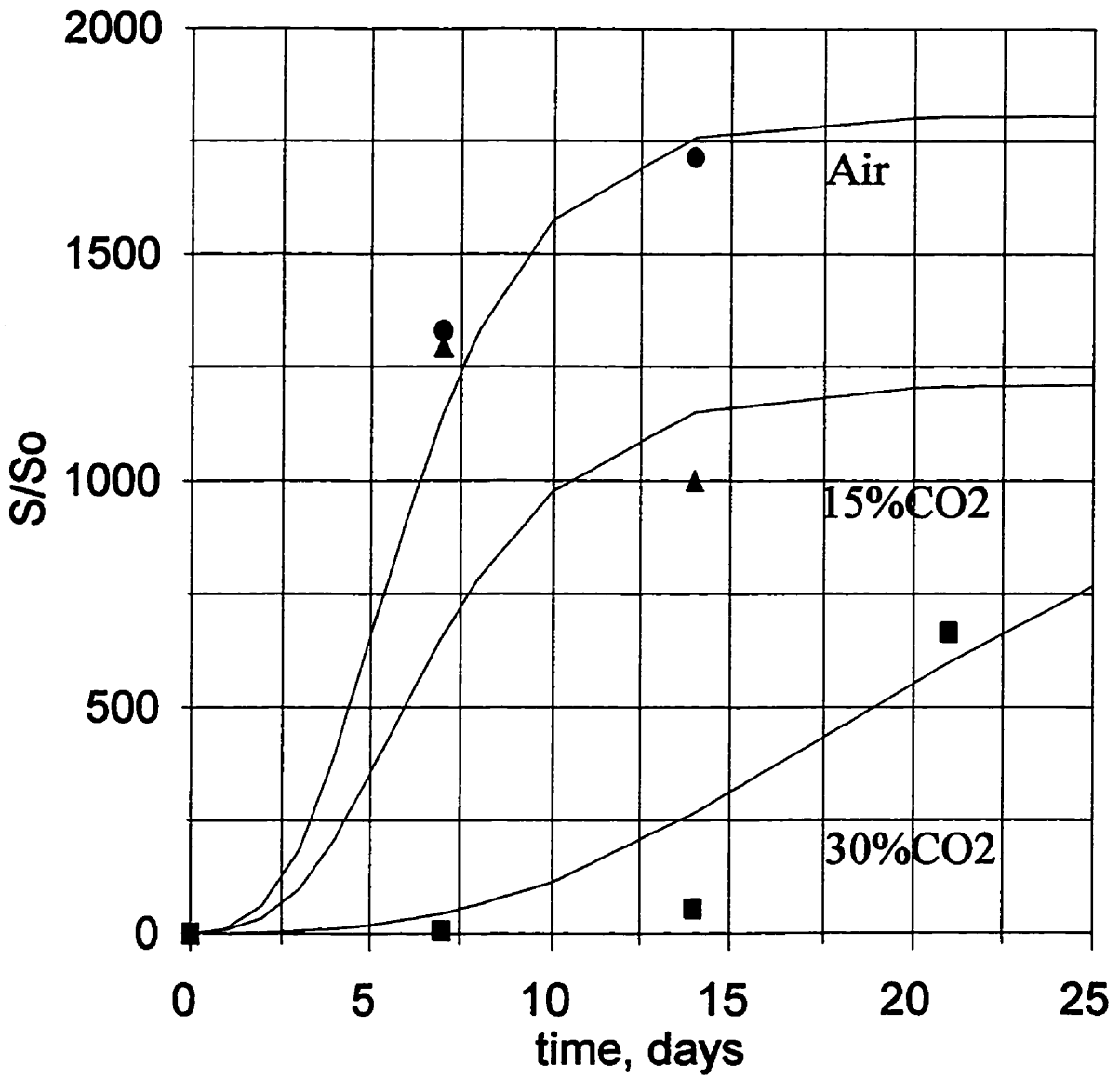

2000
1500
1000
500
0
S/So
Air
15%CO2
30%CO2
0
5
10
15
20
25
time, days

Figure 25. Growth ratio as a function of time for beef (50) stored at 11^0C.

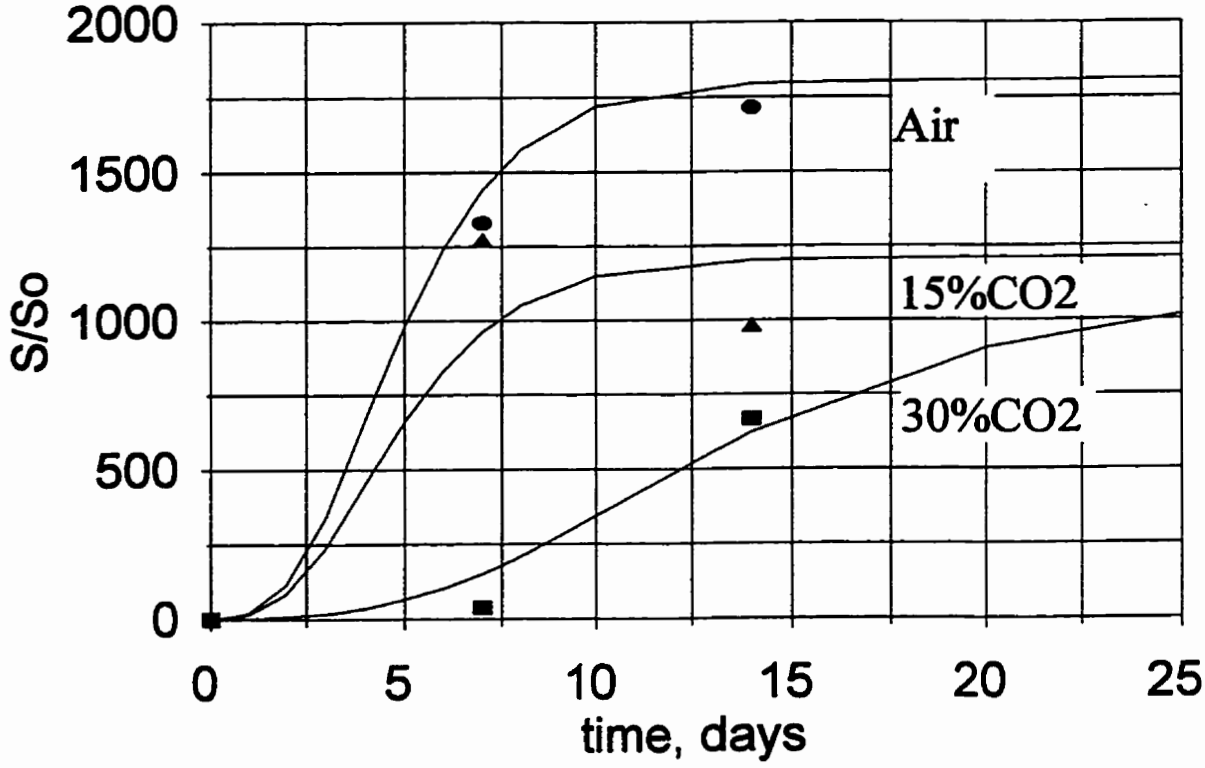

2000
1500
1000
500
0
S/So
0
5
10
15
20
25
time, days
Air
15%CO2
30%CO2

Figure 26. Growth ratio as a function of time for beef (54) stored at 11^0C.

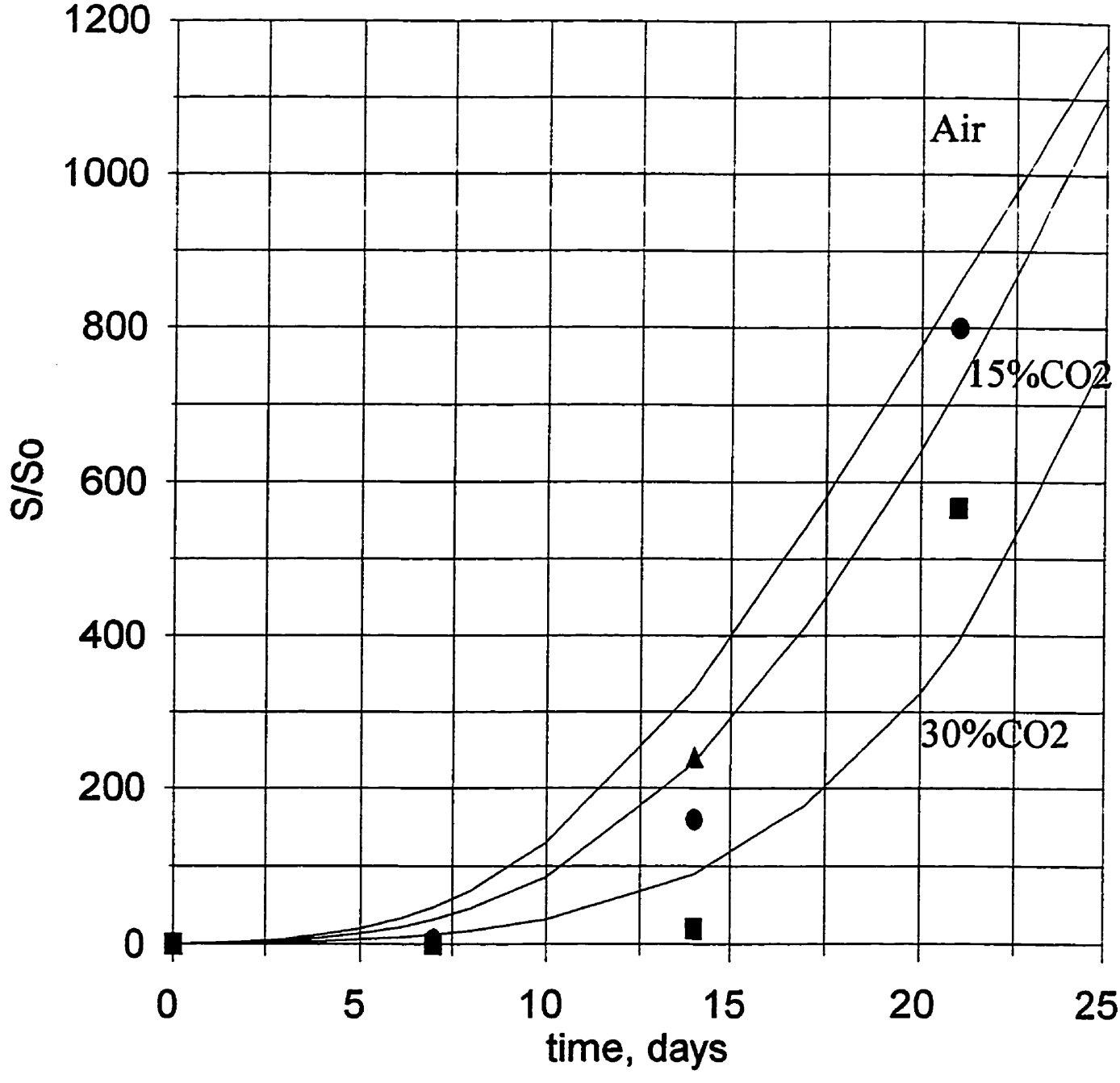

1200
1000
800
600
400
200
0
S/S0
0
5
10
15
20
25
time, days
Air
15%CO2
30%CO2

Table 11: Proportionality constants and decay in specific growth for meat of different protein content stored at various atmosphere conditions at 5^0C and 11^0C.

Type of Meat	Level of CO_2	Temp. of Storage	μ_0^*	D	Temp. of Storage	μ_0^*	D
Beef (20)	air		29	0.006		7.5	0.30
	15% CO_2		12	0.003		7.0	0.18
	30% CO_2		11	0.002		6.6	0.14
Beef (47)	air		28	0.008		7.5	0.40
	15% CO_2		12	0.003		7.1	0.35
	30% CO_2	5^0C	11	0.002	11^0C	7.1	0.11
Beef (50)	air		27	0.008		7.5	0.50
	15% CO_2		12	0.002		7.1	0.49
	30% CO_2		11	0.001		7.0	0.18
Beef (54)	air		13	0.004		7.7	0.10
	15% CO_2		12	0.002		8.1	0.08
	30% CO_2		11	0.001		10.5	0.04

For 5^0C, as in Figures 19-22, the simulation model represented the inital growth phase of the organism. Similar situation was represented for Figure 26. However, in Figures 23-27, which represent 11^0C, the simulation curve reached a plateau (that is, reached the stationary phase of the organism).

The coefficients, μ_0^* and D (of Equation 21), were combined to the various CO_2 levels and protein content. Therefore, μ_0^* in Table 11 was represented as the linear function of CO_2 in Figure 27 for 5^0C and Figure 28 for 11^0C.

$$\mu_0^* = c \times \% CO_2 + d \qquad (22)$$

where, c and d are coefficients which were found based on the best fit line. These coefficients are given in Table 12.

The decay coefficient, D, represents the linear function of CO_2. It is shown in Figure 29 and 30:

$$D = e \times \%CO_2 + f \qquad (23)$$

where, e and f are coefficients of the linear equation (as in Table 13) and,

$\%CO_2$ is the storage condition.

The coefficient c, in Equation 22, was expressed as the function of protein content and is shown in Figure 29 for 5^0C and in Figure 30 for 11^0C. The four data points within the figure indicate the various protein content. The solid line represents the best quadratic equation of the form, which can be generally expressed as:

$$y = c_1x^2 + c_2x + c_3 \qquad (24)$$

Figure 27. The proportionality constant, μ_0^*, of ground beef with various protein contents stored at 5^0C.

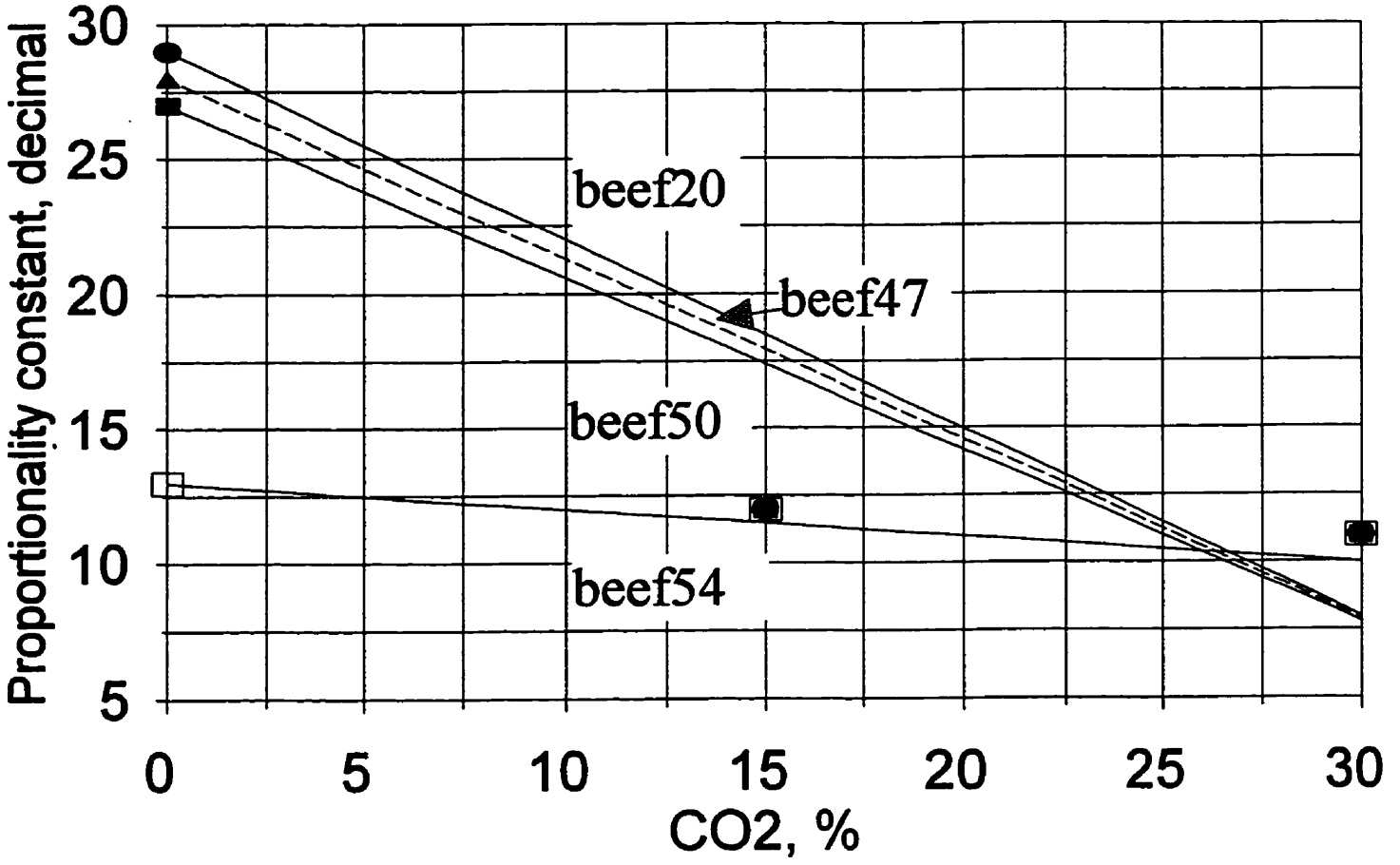

Proportionality constant, decimal
30
25
20
15
10
5
0
5
10
15
20
25
30
CO2, %
beef20
beef47
beef50
beef54

Figure 28. The proportionality constant, μ_0^*, of ground beef with various protein contents stored at 11^0C.

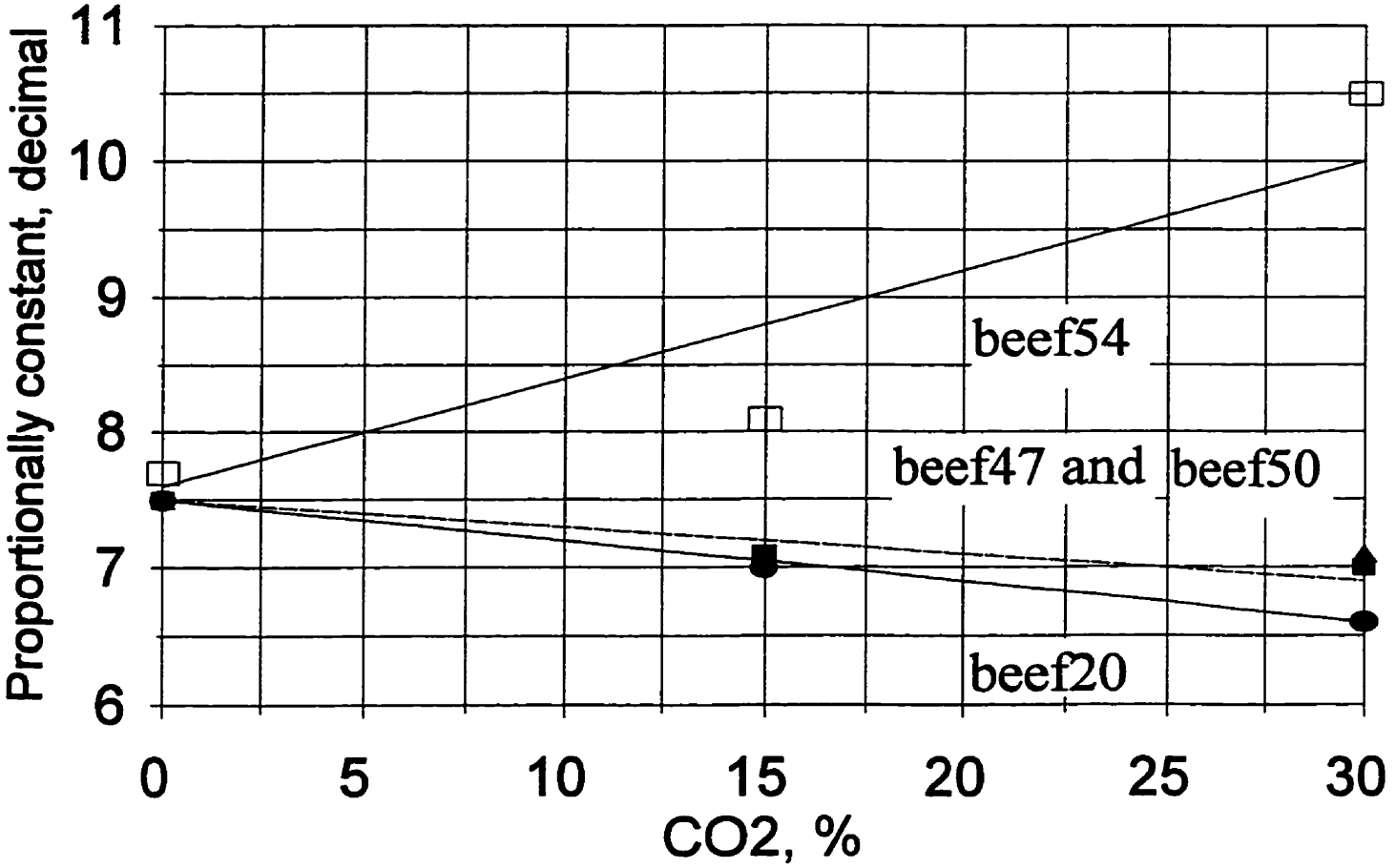

Proportionally constant, decimal
11
10
9
8
7
6
0
5
10
15
20
25
30
CO2, %
beef54
beef47 and beef50
beef20

Figure 29. The decay, D, in specific growth of *L. monocytogenes* in ground beef with various protein contents stored at 5^0C.

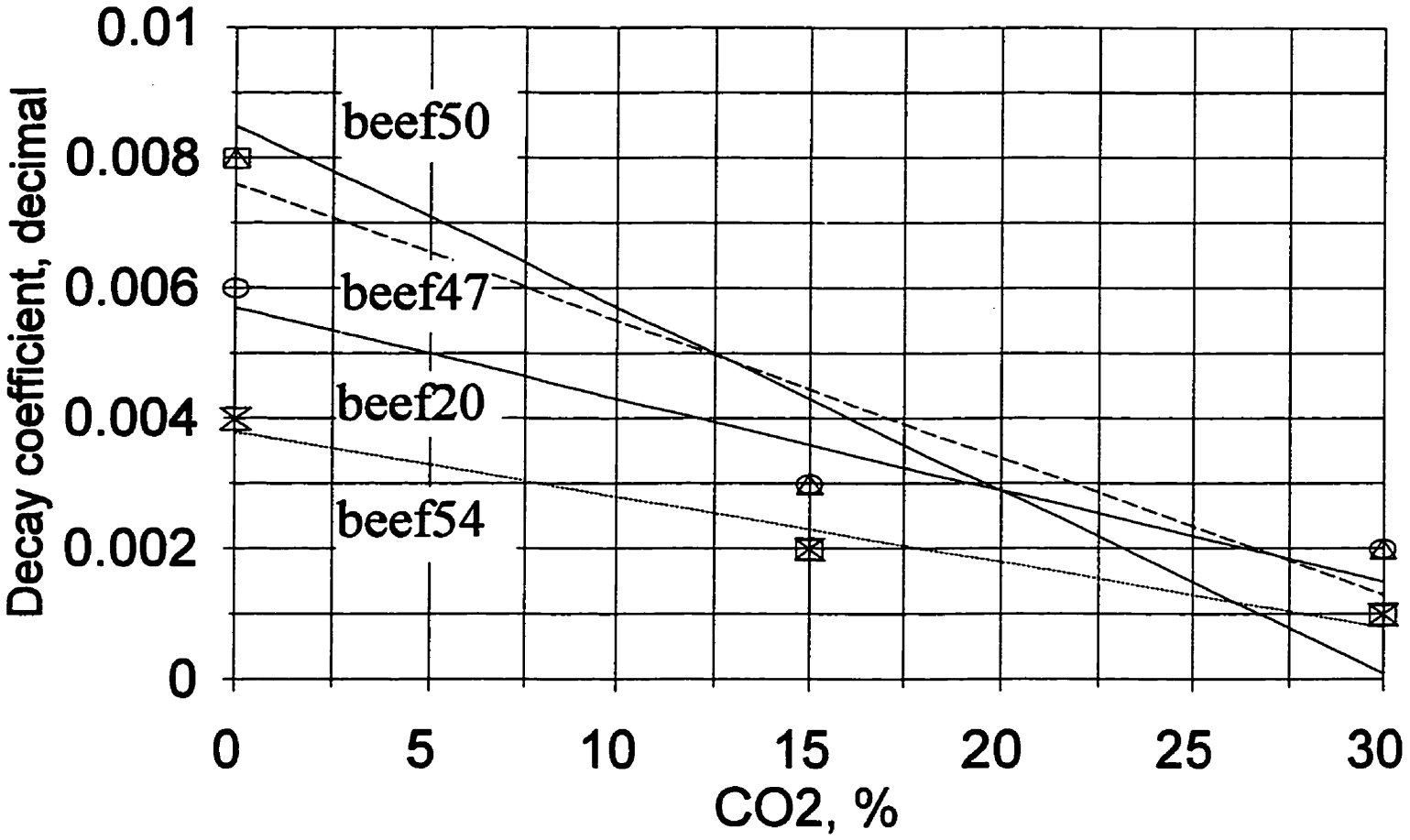

0.01
0.008
0.006
0.004
0.002
0
Decay coefficient, decimal
beef50
beef47
beef20
beef54
0
5
10
15
20
25
30
CO2, %

Figure 30. The decay in specific growth of *L. monocytogenes* in ground beef with various protein contents stored at 11^{0}C.

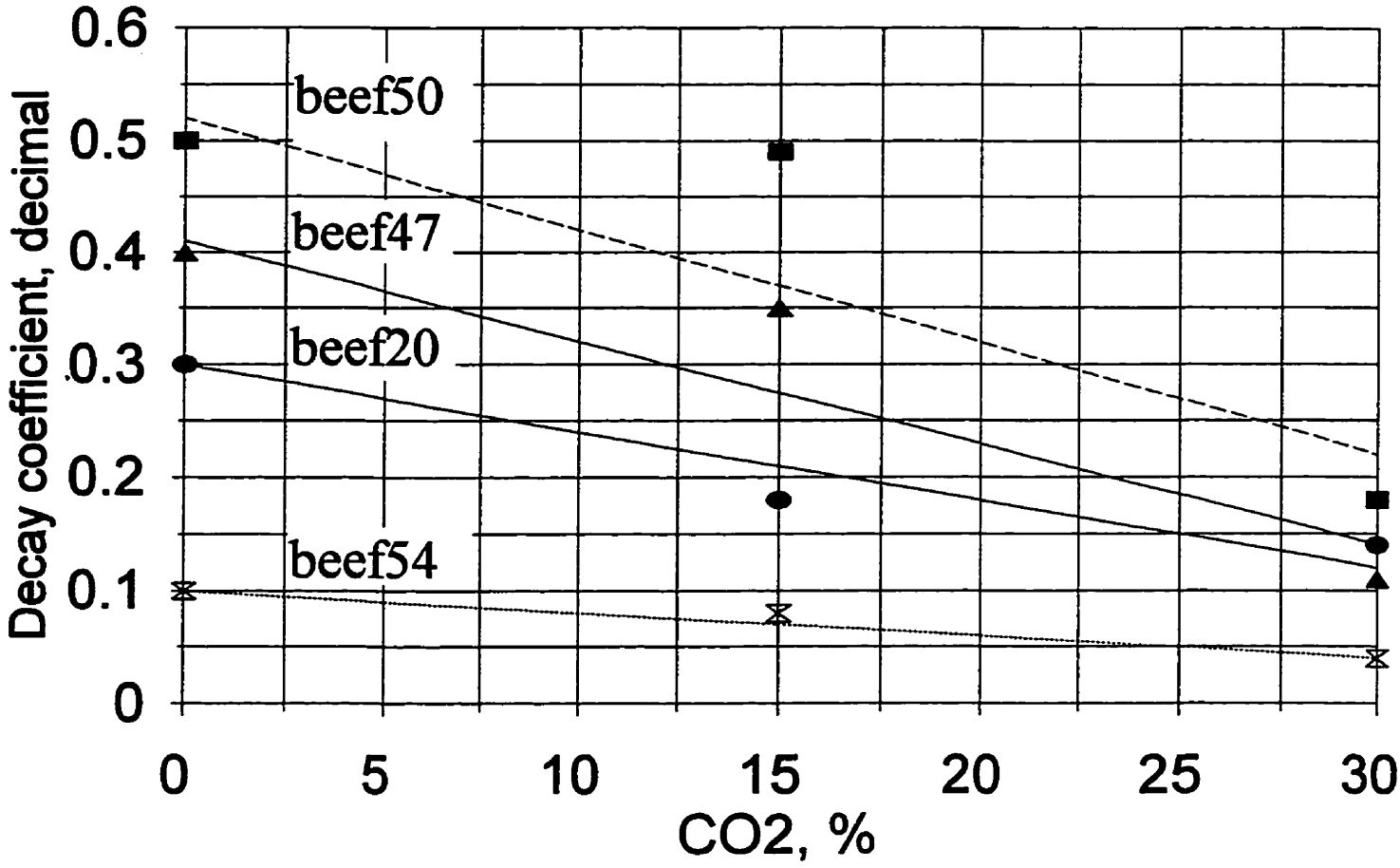

0.6
0.5
0.4
0.3
0.2
0.1
0
Decay coefficient, decimal
beef50
beef47
beef20
beef54
0
5
10
15
20
25
30
CO2, %

Figure 31. Coefficient c as a function of fat at 5^0C.

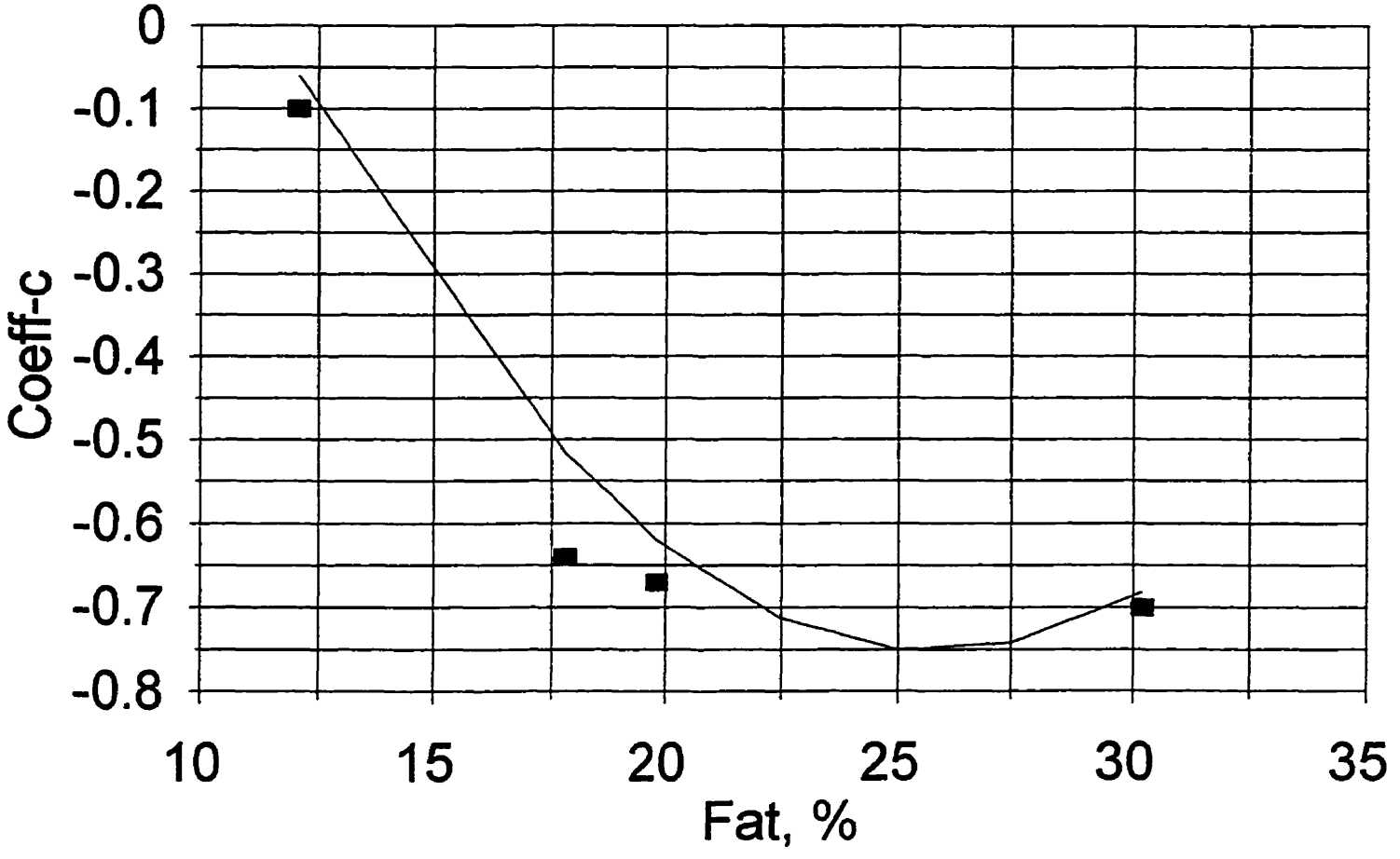

0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
Coeff-c
10
15
20
25
30
35
Fat, %

Figure 32. Coefficient c as a function of fat at 11^{0}C.

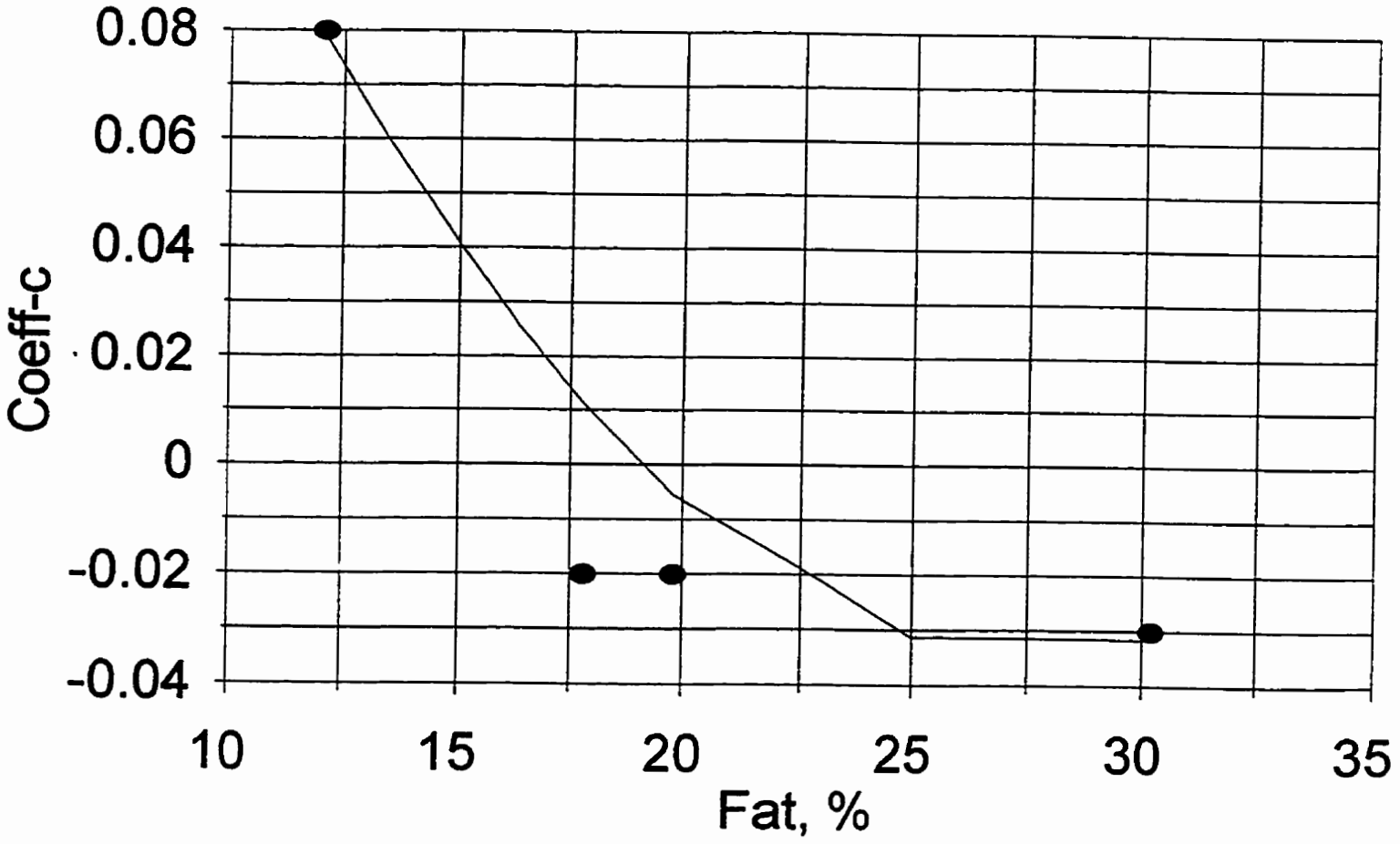

0.08
0.06
0.04
0.02
0
-0.02
-0.04
Coeff-c
10
15
20
25
30
35
Fat, %

Table 12: Coefficient c and d in specific growth for meat of different protein content stored at various atmosphere conditions at 5^0C and 11^0C.

Type of Meat	Temp. of Storage	c	d	Temp. of Storage	c	d
Beef (20)		-0.70	29		-0.03	7.5
Beef (47)	5^0C	-0.67	28	11^0C	-0.02	7.5
Beef (50)		-0.64	27		-0.02	7.5
Beef (54)		-0.10	13		0.08	7.6

Table 13: Coefficient e and f in specific growth for meat of different protein content stored at various atmosphere conditions at 5^0C and 11^0C.

Type of Meat	Temp. of Storage	e	f	Temp. of Storage	e	F
Beef (20)		-0.00014	0.0057		-0.006	0.30
Beef (47)	5^0C	-0.00021	0.0076	11^0C	-0.009	0.41
Beef (50)		-0.00028	0.0085		-0.010	0.52
Beef (54)		-0.00010	0.0028		-0.002	0.10

In this thesis, y is represented by the c coefficient and c_1, c_2 and c_3 are established based on the best fit line. The independent variable x represents the fat content. Thus, for storage conditions in 5^0C, Equation 24 became:

$$c = 0.00368F^2 - 0.19F + 1.70 \qquad (25)$$

and, for storage conditions in 11^0C:

$$c = 0.00047F^2 - 0.026F + 0.325 \qquad (26)$$

Coefficients d, e and f, in Equations 22 and 23, were best presented by quadratic equation, thus, they were expressed as quadratic functions. In Table 14 these coefficients are presented as percentages of fat.

Verification of the growth ratio

$$\frac{S}{S_o} = \exp(\mu_o^* (1 - \exp(-Dt))$$

The above derived model, with the obtained coefficients, allowed us to simulate the growth of the microorganism at 5^0C and 11^0C, in meat with fat content ranging from 12% to 30% and packaged in CO_2 content of up to 30%. The verification results of this model are presented in Figure 33 for two random experiments. The simulated conditions were as follows: inoculated beef (50) packaged in air and 15% CO_2, stored at 5^0C with the initial population at 10^5 CFU/g. The mathematical model representing the two storage conditions (15% CO_2 and air) were:

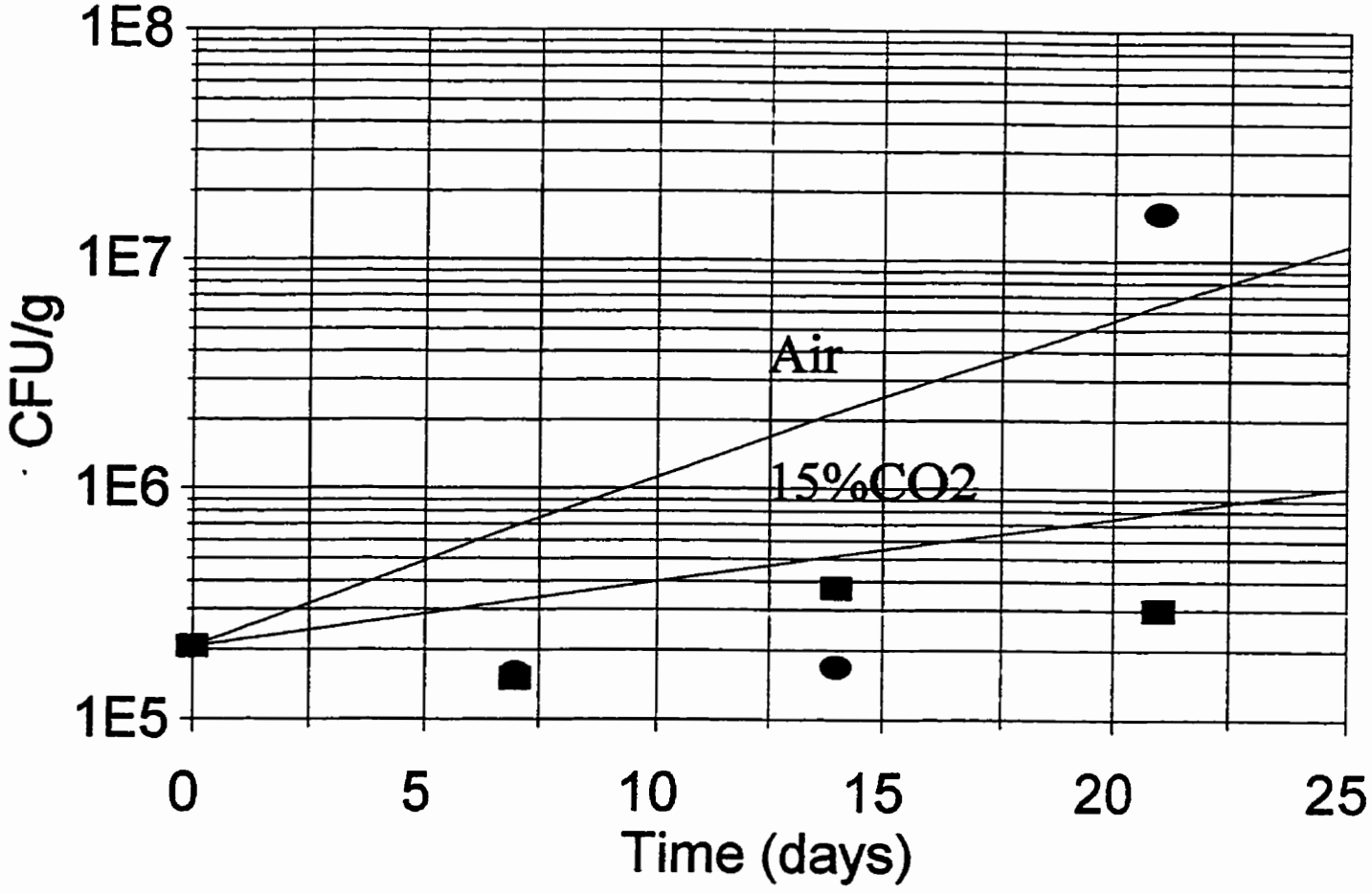
$$\frac{S}{S_o} = \exp(\mu_o^* (1 - \exp(-Dt))$$

where, $\mu_0^* = c \times CO_2 + d$, and, $D = e \times CO_2 + f$

Table 14: Values of the constants of the quadratic equation for coefficient d, e, and f.

	5^0C			11^0C		
Coefficient	c_1	c_2	c_3	c_1	c_2	c_3
D	-0.094	4.86	-32.0	0.00058	-0.03	7.88
E	0.00000142	-0.0000621	0.000443	0.000071	0.0032	0.026
F	-0.0000388	0.00174	-0.01155	-0.0035	-0.16	-1.328

Figure 33. Verification of the mathematical model, $\frac{S}{S_o} = \exp(\mu_o * (1 - \exp(-Dt))$, with *L. monocytogenes* inoculated in beef (47) packaged in air and 15% CO_2 and stored at 5^0C.


1E8
1E7
1E6
1E5
CFU/g
Air
15%CO2
0
5
10
15
20
25
Time (days)

$$c = 0.00368F^2 - 0.19F + 1.70$$

$$d = -0.094F^2 + 4.86F - 32.0$$

$$e = 0.00000142F^2 - 0.000F + 0.000443$$

$$f = -0.0000388F^2 + 0.00174F - 0.01155$$

The vertical bars in Figure 35 are associated with the experimental data points and these bars indicate 95% confidence limit. The solid lines are the simulation results. A semi-log scale was used, thus, the confidence bars are not symmetrical. The simulation results and data points were in good agreement.

Application of the growth ratio after irradiation treatment

The same coefficients derived for non-irradiated products are applicable. The growth ratio is as follows:

$$\frac{S}{S_o} = \exp\left[(\mu_o^* \left(1 - \exp\left(-D(t - t_D\right)\right)\right)\right]$$

The time delay, t_D, is as the result of irradiation. In this equation the growth ratio for the time between 0 day to 7 day is 1. This is proven experimentally as in Figures 34 to 37. It appears that there is a delay in the increase of colony forming units when the product has been subjected to 1.75 kGy. At 5^0C, t_D was generally closer to 21 days except for three cases. In these situations, the t_D was at 28, 14 and 7 days, as depicted in Figures 38, 39 and 40, respectively.

In Figures 34 to 37, an additional simulation was performed. Here, the computation was carried out with the μ_o^* values begin 10 (for beef (50)) and 12 (for beef (54)). This proved that the simulation results are very similar to that of the

experimental data. In such case, it appears that irradiation may enhance the multiplication of the organisms at 11^0C. The growth ratios reached values in the thousands, which is unusual for irradiated products when compared to the growth ratios for non-irradiated products.

Figure 34: Simulation of growth ratio as a function of time for the following conditions: $\mu_o^* = 7.5$ and 10 in air and 15% CO_2 at 11^0C.

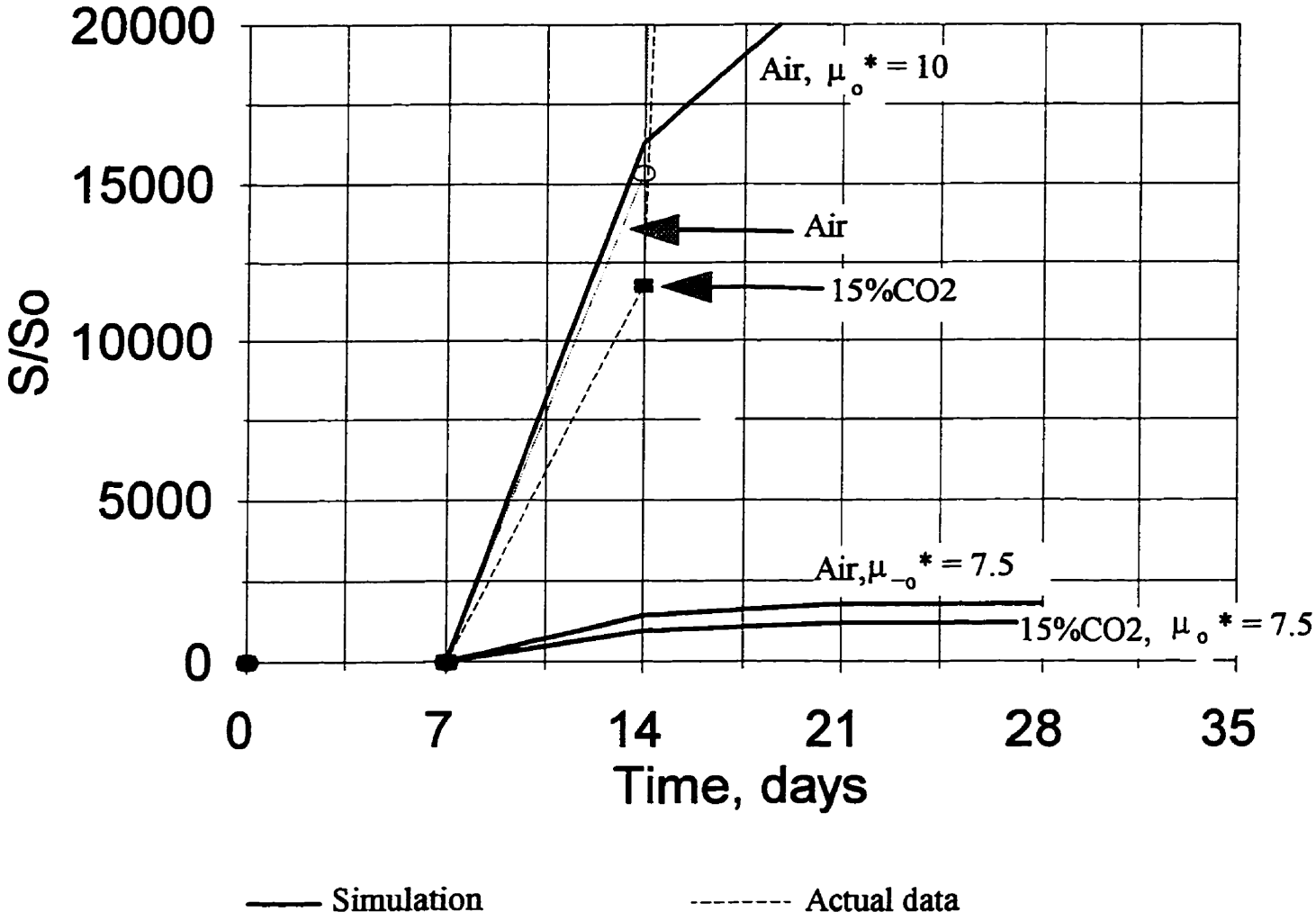

20000
15000
10000
5000
0
S/So
Air, μo* = 10
Air
15%CO2
Air,μo* = 7.5
15%CO2, μo* = 7.5
0
7
14
21
28
35
Time, days
Simulation
Actual data

Figure 35: Simulation of growth ratio as a function of time for the following conditions: $\mu_o^* = 10$ in air and 30% CO_2 at 11^0C.

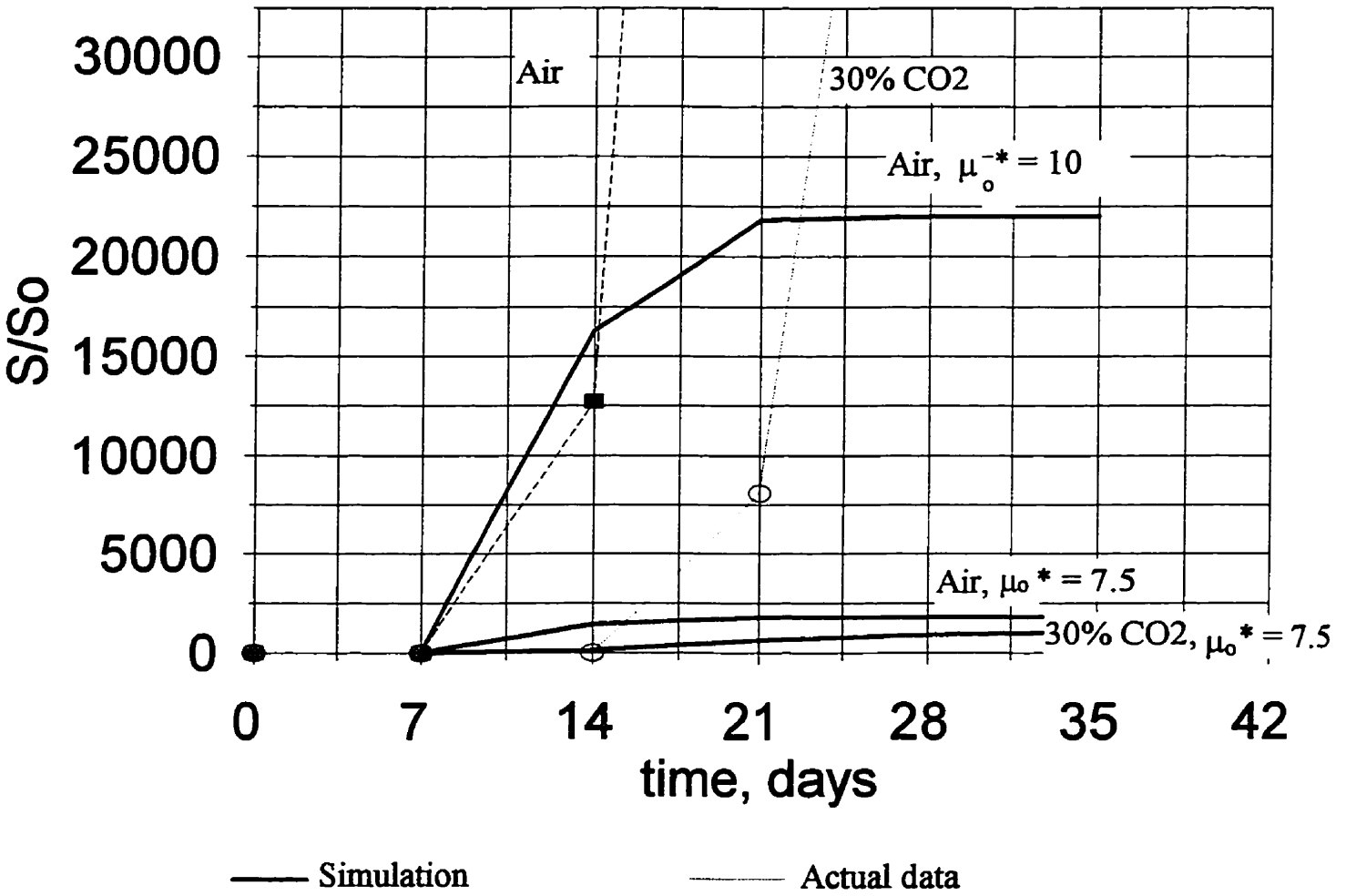

30000
25000
20000
15000
10000
5000
0
S/S0
Air
30% CO2
Air, μo* = 10
Air, μo* = 7.5
30% CO2, μo* = 7.5
0
7
14
21
28
35
42
time, days
Simulation
Actual data

Figure 36: Simulation of growth ratio as a function of time for the following conditions: $\mu_o^* = 12$ in air and 15% CO_2 11^{0}C.

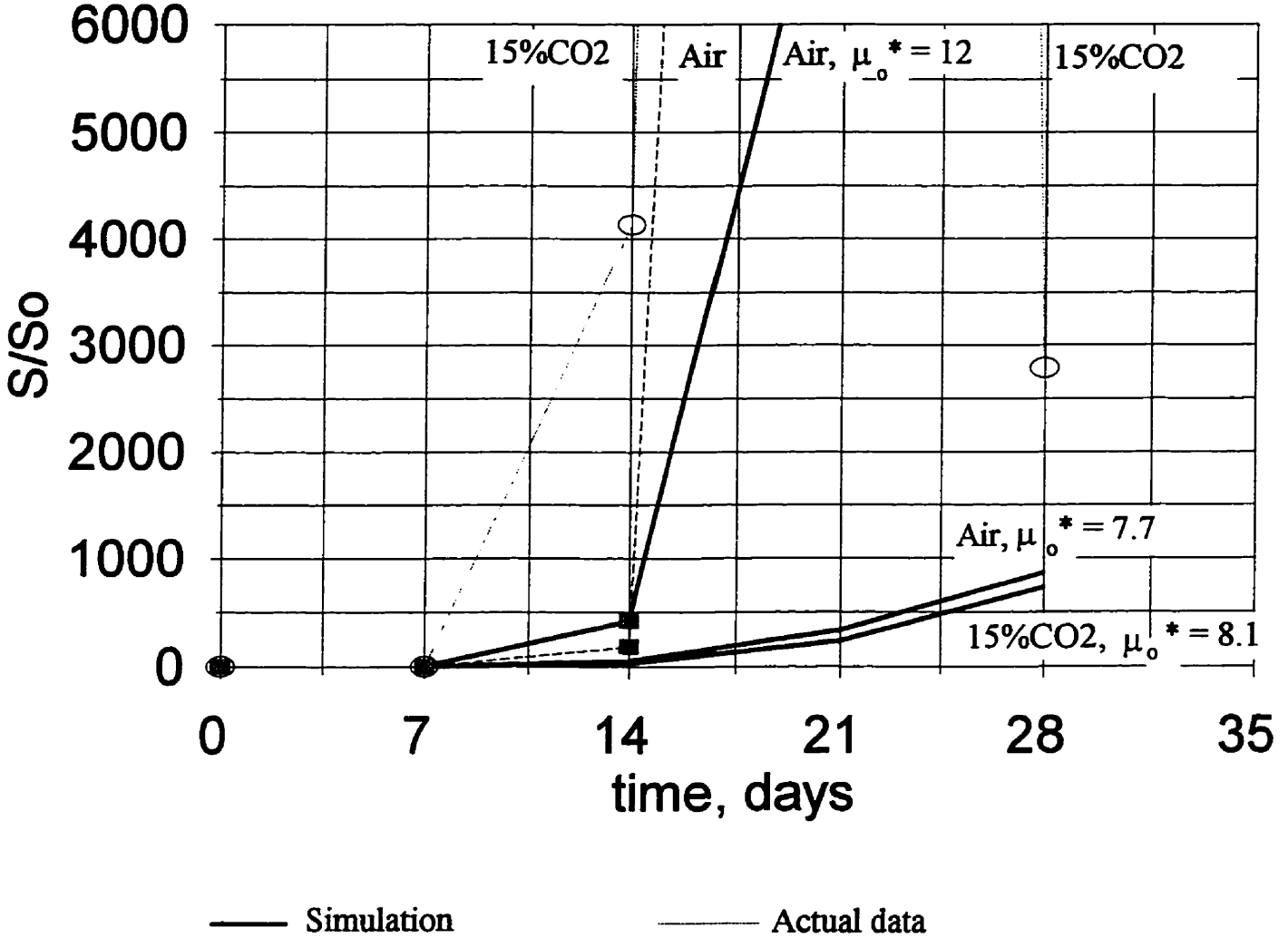

6000
5000
4000
3000
2000
1000
0
S/So
15%CO2
Air
Air, μo* = 12
15%CO2
Air, μo* = 7.7
15%CO2, μo* = 8.1
0
7
14
21
28
35
time, days
Simulation
Actual data

Figure 37: Simulation of growth ratio as a function of time for the following conditions: $\mu_o^* = 12$ in air and 30% CO_2 at 11^0C.

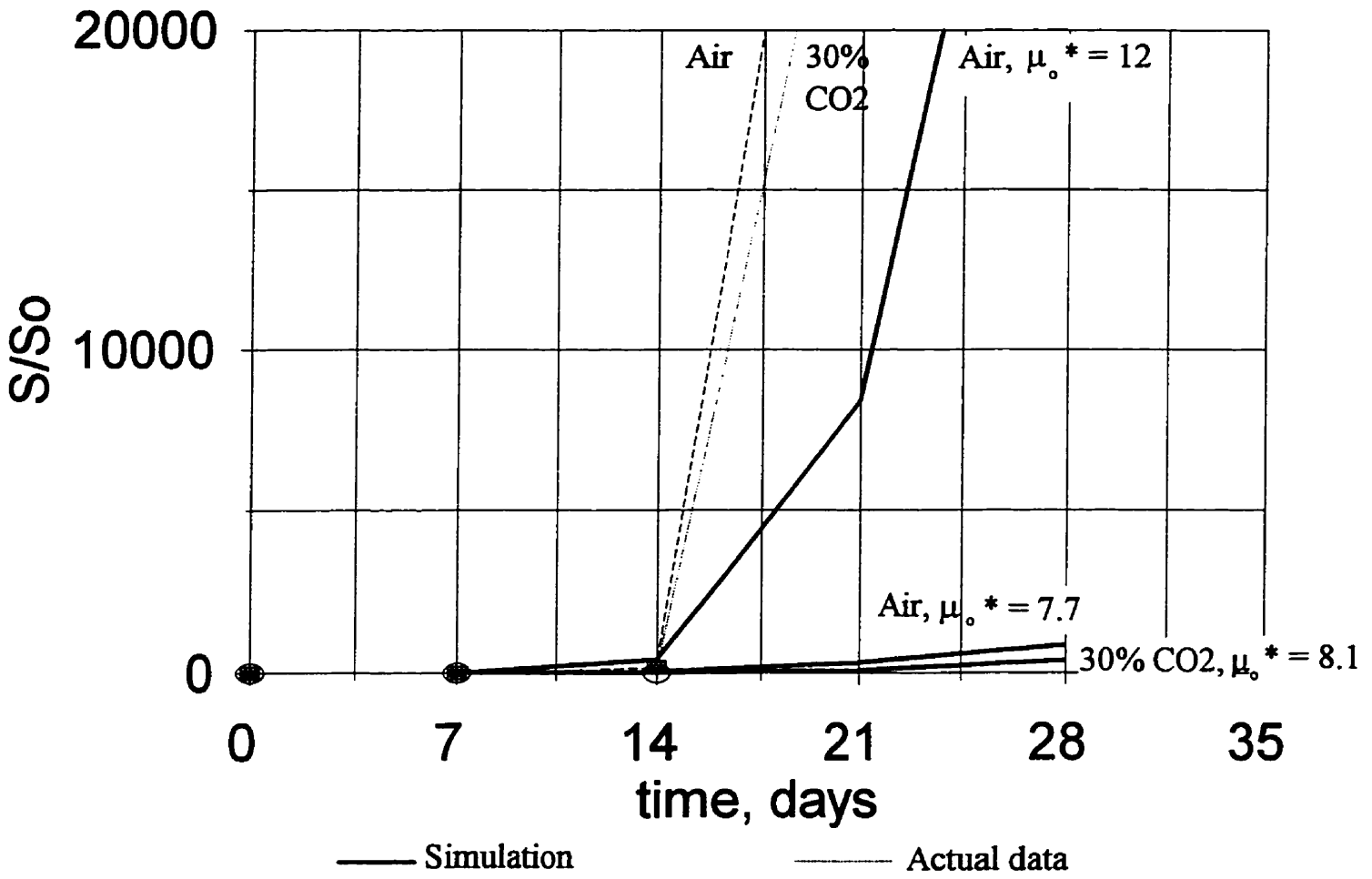

20000
10000
0
S/So
Air
30% CO2
Air, μo* = 12
Air, μo* = 7.7
30% CO2, μo* = 8.1
0
7
14
21
28
35
time, days
Simulation
Actual data

Figure 38: Simulation verses actual data of growth ratio in air and 15% CO_2 at 5^0C.

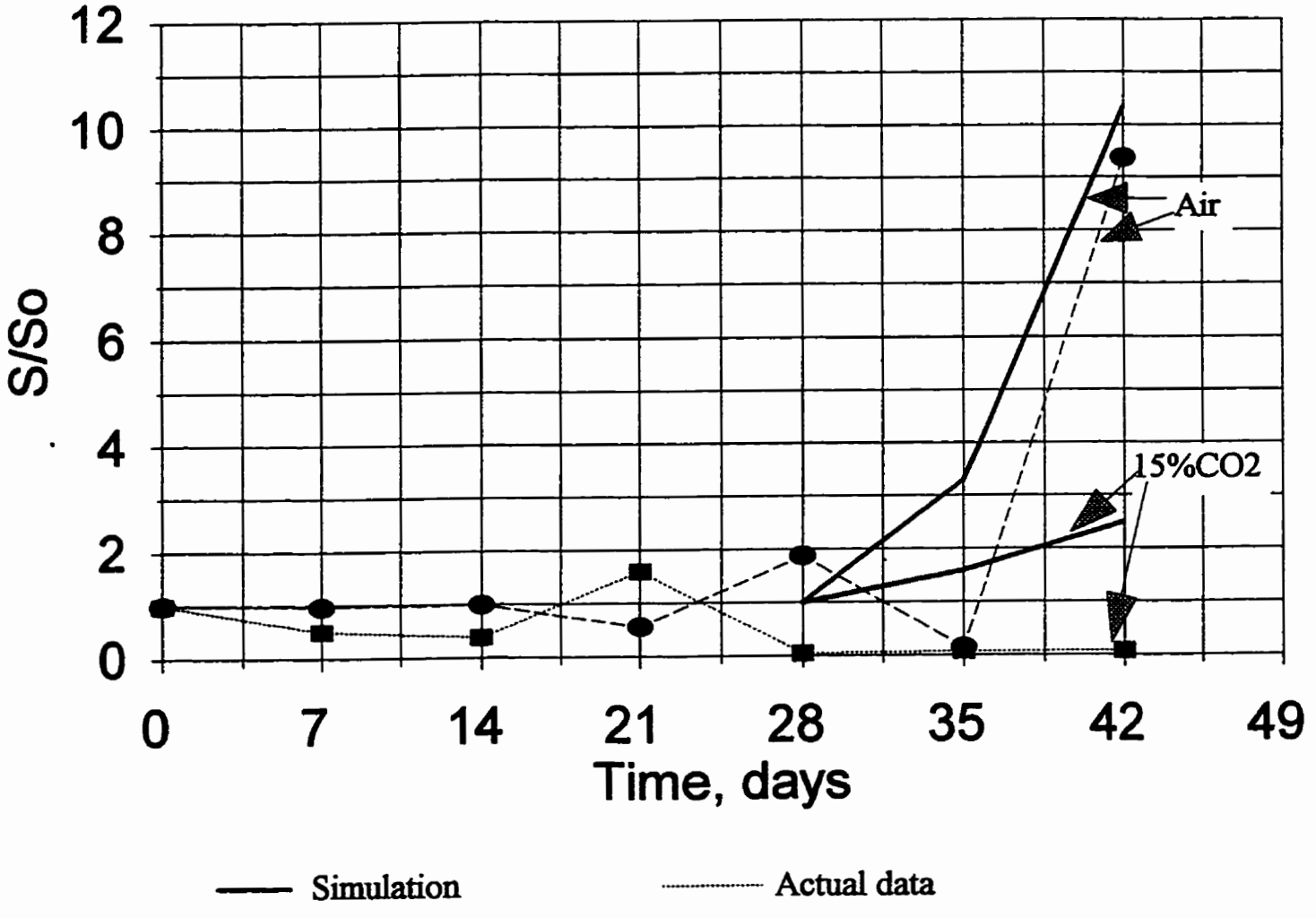

12
10
8
6
4
2
0
S/So
0
7
14
21
28
35
42
49
Time, days
Air
15%CO2
Simulation
Actual data

Figure 39: Simulation verses actual data of growth ratio in air and 30% CO_2 at 5^0C.

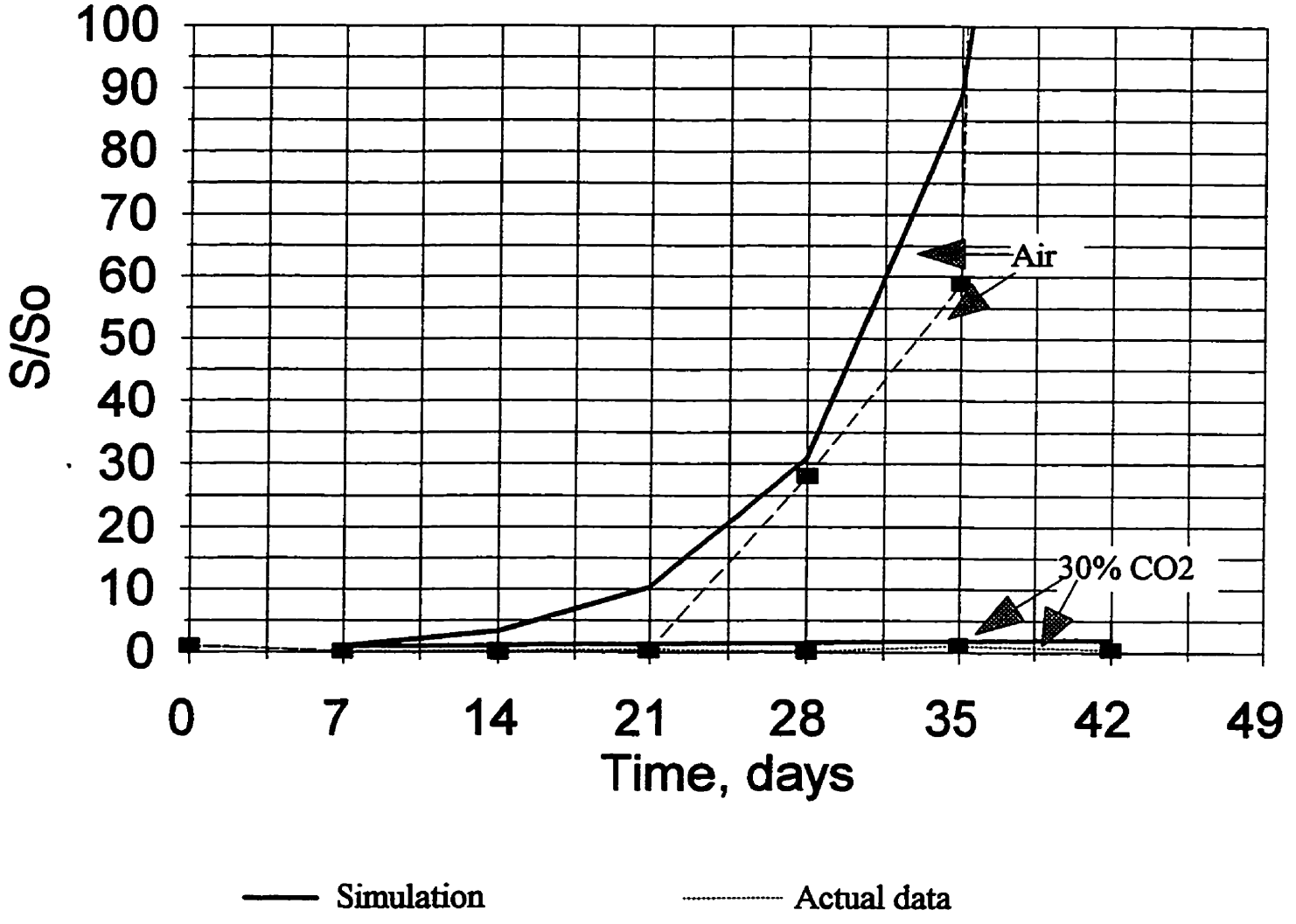
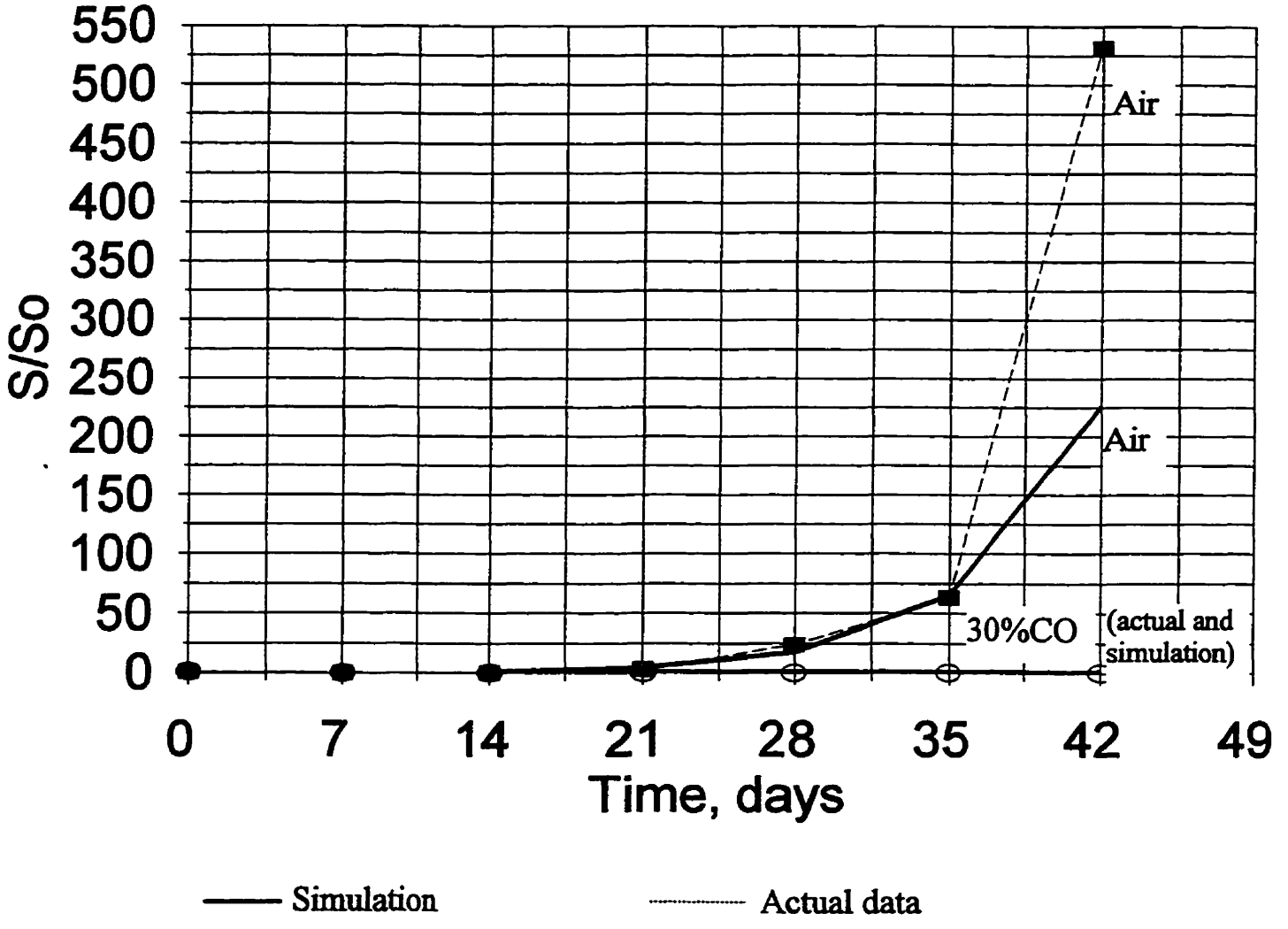

100
90
80
70
60
50
40
30
20
10
0
S/So
Air
30% CO2
0
7
14
21
28
35
42
49
Time, days
Simulation
Actual data

Figure 40: Simulation verses actual data of growth ratio in air and 30% CO_2 at 11^0C.


550
500
450
400
350
300
250
200
150
100
50
0
S/So
Air
Air
30%CO
(actual and simulation)
0
7
14
21
28
35
42
49
Time, days
Simulation
Actual data

DISCUSSION

Atmospheres containing 15% and 30% CO_2 when compared to air, appeared to affect the growth rate of *L. monocytogenes* in ground beef stored at both 5 and 11^{0}C. During the 21 d storage period at 5^{0}C, in most instances, listeriae growth in CO_2 atmospheres was approximately 1.0 to 1.5 logs lower than when packaged in air. At 11^{0}C, the difference in listerial growth between CO_2 and air was approximately 0.5 logs, with the exception of Beef (47); in this case the difference was observed by day 21. Similarly, by applying mathematical modeling, the growth ratio of *L. monocytogenes* increased as the level of CO_2 decreased. The growth ratio demonstrates the effect of the atmosphere on the growth of the organism. The increase in growth ratio of this organism, as the level of CO_2 decreased, was observed at both 5 and 11^{0}C. In general, the growth ratio was greater in air than in 15% CO_2. The smallest growth ratio was given in carbon dioxide atmospheres of 30%. These results are contrary to those of Berrang et al., (1989) who reported that the growth of *L. monocytogenes* in asparagus, cauliflower and broccoli with 3-10% CO_2 was not affected at either 4 or 15^{0}C. This difference may be due to the fact that the lowest level of CO_2 applied in this study was 15%, whereas, Berrang et al. (1989) used 10% CO_2 as their highest level. Furthermore, different substrates were used in both studies (ground beef versus vegetables).

It is well accepted that the effectiveness of CO_2 in inhibiting microbial growth decreases as the fat content of the product increases. Baker et al. (1985),

for example, demonstrated that the survival of *S. typhimurium* and *Cl. sporogenes* stored under CO_2 was greater in broth as compared to chicken meat. Although this trend is present in this study, it is not easily detected. However, if the growth ratio of Beef (20) and Beef (54) are compared, a slight decrease in growth ratio ca. from 2.5 to 1.75 and ca. from 2.0 to 1.4 for 15% and 30% CO_2, respectively can be observed. This observation may be attributed to the increased effectiveness of CO_2 as the fat content of the ground beef decreased.

Irradiation (1.75 kGy) was observed to decrease the initial bacterial load by ca. 2 logs in packaged beef containing either air or CO_2. Grant and Patterson (1991) reported that one strain of *L. monocytogenes* was reduced by two log cycles, while another strain was reduced by three log cycles. Both strains were inoculated into minced pork and then subjected to 1.75 kGy. Thayer (1995) also stated that a dose of 1.5 kGy reduced the viable population of listeriae by at least 10^2 CFU/ ml and that a dose of 2.5 kGy should inactivate at least 10^3 CFU/ ml.

Packaging with CO_2 when combined with irradiation, resulted in an overall slow down of growth. For example, generally there appeared to be ca. a 2 log difference by day 42, in the growth level of listeriae between air and CO_2 packaged (5^0) ground beef. This trend suggests that the sensitivity of the organism to irradiation may be increased when treated under CO_2 (Patterson, 1988). Hastings et al. (1986) suggested that irradiation and CO_2 may act synergistically to enhance the lethal effect. However, there appeared to be no difference in the growth levels of listeriae between

15 and 30% CO_2 packaged ground beef when stored under the same temperature, after irradiation treatment.

After irradiation treatment, a slight decrease in listeriae was observed at ca. 7 to 14 d of storage. This decline in population may represent a lag period during which survivors undergo repair from injury following irradiation treatment. Patterson et al. (1993) also reported that the lag phase of *L. monocytogenes* at 6^0C following radiation (2.5 kGy) was extended from 1 to 18 d. As a result, the researcher assumed that low levels of survivors would not be a problem during the normal refrigerated shelf-life of the product. This observation supports the notion that synergistic effects from combination treatments may enhance the preservation of food.

Increasing the protein content of the menstruum should provide protection to the organism against irradiation (El-Shenawy et al.,1989). These researchers found that although irradiation injury of *L. monocytogenes* may occur in either broth or raw ground beef, the resistance of this bacterium to radiation treatment was greater in beef. Their results were similar to those obtained by Mulder (1982). The results obtained in this study, however, did not reveal this finding. After 42 d of storage the listeriae population detected in Beef (20) and Beef (54) was ca. 10^2 CFU/g and 10^1 CFU/g, respectively when packaged in 30% CO_2 at 5^0C. Similar findings were also observed when the product was packaged in air; in this case the number of organisms detected in Beef (20) and Beef (54) was ca. 10^5 CFU/g and ca. 10^3 CFU/g, respectively. Although statistical comparisons were not carried out, the

inconsistency of these finding as compared to results reported by El-Shenawy et al. (1989) may indicate that the protein difference between the ground beef samples was not large enough to exhibit this trend. Furthermore, the samples used by El-Shenawy et al. (1989) consisted of used a liquid and a solid; this difference in itself may enhance the selective survival of the organism. Furthermore, a comparsion of the growth ratio would not permit for an accurate conclusion since the number of survivors were too low.

The inhibitory effect of CO_2 was observed to decrease as the storage temperature increased. This was also observed in products which were irradiated. This accords with previous studies in that the effectiveness of CO_2 is reduced at higher temperatures (Finne, 1982). Enfors and Molin (1981) studied the effect of 50% CO_2 on the growth of *Ps. Fragi* in muscle food and found that the growth rate was inhibited about 30% at 35^0C, 50% at 30^0C and, 90% at 5^0C. This effect was explained by increasing solubility of CO_2 with decreasing temperature (Knoche, 1980; Ogrydziak and Brown, 1982). The results of this study emphasize the need for properly controlled refrigerated storage if maximum benefits are to be derived from the combination of MAP and irradiation to control the growth of microorganisms. In particular, since *L. monocytogenes* is well adapted to grow at 4 to 5^0C, temperature is a crucial factor.

Qualitative analysis indicated that regardless of the storage treatment, haemolytic activity in *Listeria* was still observed. This observation indicated that although the synergistic effect of MAP and irradiation may contribute to a decrease in

the number of surviving organisms, it does not appear to reduce some of the virulence properties of the organism.

Quantitative analyses in relation to pathogenicity in listeriae revealed that the combination treatment involving CO_2 and irradiation resulted in a decrease in the rate of death of the chick embryos. This appears to be due to the reduced number of viable microorgansims.

The results also showed that an increase in storage temperature for listeriae in meat increased the death rate of the inoculated chick embryos. In effect, the synergistic effect of the MAP and irradiation appears to be more effective under refrigeration temperatures of 5^0C than at 11^0C. Thayer (1995) reported that verotoxin, produced by *E. coli* found in lean beef which was irradiated at 0.75 kGy when stored at 35^0C for 20 h, whereas, when stored at 5^0C, no toxin production was found.

Avery and Buncic (1997) studied the pathogenicity of chick embryos inoculated with meat isolates of *L. monocytogenes*. They found that the average mortality rate of embryos decreased from 98.7% when inoculated with fresh cultures (1.5 – 2.5 $\log_{10}$ CFU per egg) as compared to 68.0% when cultures which were stored at 4^0C for 4 weeks. In the present study, it was observed that the mortality rate decreased from 100% when fresh cultures of listeriae were used (10^3 CFU per egg) as compared to 90% when cultures which were stored at 5^0C for 28 d (from meat packaged in air). Furthermore, it was observed that with inocula greater than 10^3 CFU, the death rate of the embryos was 100% regardless of the storage period.

In this study, over the 42 d storage period, growth greater than 10^1 CFU/g listeriae was never achieved when the samples were subjected to MAP and irradiation. Since a longer period of time may be required for the organism to reach a level of 10^3 CFU/g, a further study may be required to demonstrate the impact of the combination treatment on the hemolysin production by this organism.

The mathematical model, $\frac{S}{S_o} = \exp(\mu_o^* (1 - \exp(-Dt))$, is derived from the experimental data generated by this study. Fat (protein) content, storage temperature, and atmospheric conditions were the parameters which were applied to derive this equation. The different growth ratios, $\frac{S}{S_o}$, indicate that there is an impact of storage temperature, fat content, gas mixtures and irradiation on the growth of the organism. Fat alone may have an effect on the difference in the growth ratio. It is well established that pathogens such as Salmonella when suspended in buffer, broth or mechanically deboned chicken (Thayer et al., 1990) and *L. monocytogenes* when it is suspended in buffered saline or poultry meat (Patterson, 1989) have different radiation sensitivities. Due to the low survival rate of the organism after being subjected to MAP and irradiation, $\frac{S}{S_o}$ was not determined in this study.

Verification of this model can be carried out as long as the parameters applied are within the conditions used in this study. That is, the growth of the microorganism is between 5^0C and 11^0C, in meat with protein content ranging from 12% to 36%

protein content and packaged in CO_2 content of up to 30%. The verification results carried out in this study maintain data points that are of 95% confidence limit.

The mathematical model, $\frac{S}{S_o} = \exp\left[(\mu_o^* (1 - \exp(-D(t - t_D)))\right]$, is also derived from the experimental data generated by this study. It, however, takes into consideration the delay of growth of the organism as the result of irradiation treatment. Generally, the time delay of the product was approximately 21 days when stored at 5^0C. However, there were situations in which the time delay was at 7, 14 and 28 days.

SUMMARY AND CONCLUSIONS

Storage in modified atmospheres of 15:85% and 30:70% CO_2:N_2 did not affect the growth rate of *L. monocytogenes* at storage temperatures of 5 and 11^{0}C. Radurization was more effective in extending the shelf-life since it was able to decrease the initial bacterial load. This decline may represent a lag period in order for survivors to undergo repair from injury following irradiation treatment.

Products which has been irradiated with CO_2 and stored at 5^{0}C resulted in slower growth of *Listeria* as compared to products which has been irradiated with air and stored at 5^{0}C. Yet, there appeared to be no difference in the growth levels between 15 and 30% CO_2 products when stored under the same temperature after irradiation treatment.

Protein content of the ground beef was observed to have an effect on the growth of *L. monocytogenes*. It was found the growth of the organism decreased by ca. 1 log when the media contained 20% vs. 54% protein content. This was observed in both MAP conditions; with or without the application of irradiation; and at storage temperature of both 5 and 11^{0}C.

Inhibitory effect of CO_2 decreases as the storage temperature increase. This was also maintained with the product has been subjected to radurization. Furthermore, this study demonstrated that *L. monocytogenes* has the ability to survive under storage temperature condition of 5^{0}C. Therefore, the safety concern for foodborne illness resulting from ingestion of *Listeriae* would exist if the initial load of the product

contained this organism even though the product has been subjected to the combined treatment protocol of MAP and irradiation.

Although the synergistic effect of MAP and irradiation does decrease the initial load of the microorganism, it does not eliminate it. When qualitative hemolysis evaluation was carried out on the organisms subjected to this combined treatment protocol, it was observed that the virulence properties of the organism were not affected.

Quantitative analyses of pathogenicity in *L. monocytogenes* was demonstrated when the product was subjected to the combined treatment protocol of MAP and irradiation. This treatment protocol decreases the rate of death of chick embryos as compared to a treatment protocol consisting only of MAP. Furthermore, the synergestic effect of MAP and irradiation was demonstrated to be more effective, in decreasing the death rate of the chick embryos, at 5^0C as compared to at 11^0C.

The ability to formulate of a mathematical model from the experimental data generated by this study was demonstrated. The parameters used to derive the equation included fat (protein) content, storage temperature and atmospheric conditions. The model, $\frac{S}{S_o} = \exp(\mu_o^* (1 - \exp(-Dt))$, indicate that there is an impact of storage temperature, fat content, gas mixtures and irradiation on the growth of the organism.

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Appendix table 1: Survival and/or growth of *L. monocytogenes* in packaged beef (20) containing either 15%:85% CO_2:N_2 or air.

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
15%:85% CO_2:N_2 / 5	0	15.9×10^5	39.0×10^5	39.0×10^5
		20.0×10^5	37.0×10^5	32.0×10^5
		19.4×10^5	19.0×10^5	22.0×10^5
	7	22.0×10^5	30.0×10^4	30.0×10^4
		22.0×10^5	40.0×10^4	40.0×10^4
		18.0×10^5	70.0×10^4	20.0×10^4
	14	90.0×10^4	30.0×10^4	40.0×10^4
		10.0×10^5	17.0×10^4	85.0×10^4
		12.0×10^5	40.0×10^4	85.0×10^4
	21	13.0×10^5	17.0×10^5	54.0×10^5
		10.1×10^5	11.0×10^5	38.0×10^5
		11.8×10^5	14.0×10^5	45.0×10^5
15%:85% CO_2:N_2 / 11	0	15.9×10^5	39.0×10^5	39.0×10^5
		20.0×10^5	37.0×10^5	32.0×10^5
		19.4×10^5	19.0×10^5	22.0×10^5
	7	2.5×10^8	1.2×10^8	1.6×10^8
		2.7×10^8	1.7×10^8	2.5×10^8
		2.3×10^8	1.6×10^8	2.1×10^8
	14	1.3×10^8	1.8×10^8	1.6×10^8
		1.8×10^8	1.9×10^8	1.4×10^8
		1.4×10^8	1.8×10^8	1.7×10^8
Air / 5	0	2.1×10^5	2.3×10^5	1.8×10^5
		2.6×10^5	1.8×10^5	1.6×10^5
		20.0×10^5	2.3×10^5	1.7×10^5
	7	19.0×10^5	24.0×10^5	1.0×10^5
		47.0×10^5	27.0×10^5	1.0×10^5
		42.0×10^5	28.0×10^5	1.0×10^5
	14	2.5×10^5	7.4×10^5	11.3×10^5
		4.0×10^4	8.4×10^5	10.1×10^5
		3.2×10^5	6.5×10^5	12.5×10^5
	21	82.0×10^5	42.0×10^6	61.0×10^6
		62.0×10^5	53.0×10^6	59.0×10^6
		70.0×10^6	53.0×10^6	60.0×10^6
Air / 11	0	2.1×10^5	2.3×10^5	1.8×10^5
		2.6×10^5	1.8×10^5	1.6×10^5
		20.0×10^5	2.3×10^5	1.7×10^5
	7	3.7×10^7	6.0×10^6	13.9×10^7
		3.9×10^7	7.0×10^6	10.7×10^7
		5.7×10^7	1.3×10^7	7.6×10^7
	14	40.3×10^8	49.0×10^8	20.0×10^8
		10.4×10^8	37.0×10^8	80.0×10^7
		30.2×10^8	40.4×10^8	25.0×10^8

Appendix table 2: Survival and/or growth of *L. monocytogenes* in packaged beef (20) containing either 15%:85% CO_2:N_2 or air followed by irradiation at 1.75 kGy.

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
15%:85% CO_2:N_2 / 5	0	30.0×10^2	40.0×10^2	50.0×10^2
		30.0×10^2	50.0×10^2	50.0×10^2
		40.0×10^2	90.0×10^2	48.0×10^2
	7	2.0×10^2	3.0×10^2	10.0×10^2
		1.0×10^2	5.0×10^2	20.0×10^2
		1.0×10^2	2.0×10^2	50.0×10^2
	14	1.0×10^2	1.0×10^2	10.0×10^2
		2.0×10^2	3.0×10^2	20.0×10^2
		3.0×10^2	1.0×10^2	30.0×10^2
	21	1.4×10^3	1.0×10^2	7.5×10^2
		1.3×10^3	3.0×10^1	7.4×10^2
		1.6×10^3	1.0×10^2	7.6×10^2
	28	4.0×10^1	10.0×10^1	40.0×10^1
		10.0×10^1	10.0×10^1	20.0×10^1
		2.0×10^1	3.0×10^1	10.0×10^1
	35	20.0×10^1	10.0×10^1	10.0×10^1
		60.0×10^1	20.0×10^1	50.0×10^1
		50.0×10^1	30.0×10^1	10.0×10^1
	42	40.0×10^1	10.0×10^1	40.0×10^1
		90.0×10^1	10.0×10^1	40.0×10^1
		50.0×10^1	40.0×10^1	40.0×10^1
15%:85% CO_2:N_2 / 11	0	30.0×10^2	40.0×10^2	50.0×10^2
		30.0×10^2	50.0×10^2	50.0×10^2
		40.0×10^2	90.0×10^2	48.0×10^2
	7	70.0×10^2	30.0×10^2	30.0×10^2
		60.0×10^2	70.0×10^2	30.0×10^2
		30.0×10^2	60.0×10^2	45.0×10^2
	14	51.8×10^5	3.8×10^5	24.6×10^5
		38.0×10^5	3.7×10^5	24.6×10^5
		41.8×10^5	3.6×10^5	24.5×10^5
	21	1.5×10^6	1.1×10^6	1.4×10^6
		1.4×10^6	1.1×10^6	1.5×10^6
		1.1×10^6	1.4×10^6	1.5×10^6
	28	1.5×10^8	1.8×10^8	4.0×10^7
		7.0×10^7	9.0×10^7	4.0×10^7
		1.2×10^8	1.3×10^8	6.0×10^7
Air / 5	0	1.0×10^2	1.0×10^2	2.0×10^2
		2.0×10^2	3.0×10^2	7.0×10^2
		3.0×10^2	1.0×10^2	2.5×10^2
	7	1.0×10^2	1.0×10^2	2.0×10^2
		1.0×10^2	4.0×10^2	2.4×10^2
		3.0×10^2	5.0×10^2	2.4×10^2
	14	1.0×10^2	1.0×10^2	3.5×10^2
		5.0×10^2	3.5×10^2	1.5×10^2

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
		3.0×10^2	1.5×10^2	2.5×10^2
	21	1.0×10^2	1.0×10^2	1.4×10^2
		1.0×10^2	1.0×10^2	1.5×10^2
		3.0×10^2	1.4×10^2	1.3×10^2
	28	20.0×10^2	10.0×10^2	80.0×10^2
		70.0×10^2	30.0×10^2	40.0×10^2
		50.0×10^2	60.0×10^2	60.0×10^2
	35	1.0×10^1	2.0×10^1	6.0×10^1
		1.0×10^1	2.0×10^1	6.0×10^1
		3.0×10^1	9.0×10^1	4.0×10^1
	42	24.0×10^3	14.0×10^3	16.0×10^3
		41.0×10^3	24.0×10^3	16.0×10^3
		28.0×10^3	29.0×10^3	19.0×10^3
Air / 11	0	1.0×10^2	1.0×10^2	2.0×10^2
		2.0×10^2	3.0×10^2	7.0×10^2
		3.0×10^2	1.0×10^2	2.5×10^2
	7	3.1×10^4	70.0×10^3	60.0×10^3
		3.0×10^4	1.3×10^4	90.0×10^3
		4.7×10^4	70.0×10^3	40.0×10^3
	14	1.1×10^5	1.9×10^5	1.5×10^5
		1.2×10^5	1.9×10^5	1.5×10^5
		1.0×10^5	2.1×10^5	1.5×10^5
	21	2.3×10^6	1.0×10^6	1.5×10^6
		1.6×10^6	1.1×10^6	1.5×10^6
		2.1×10^6	1.1×10^6	1.5×10^6
	28	70.0×10^7	1.0×10^7	30.0×10^7
		2.0×10^7	3.0×10^7	30.1×10^7
		8.0×10^7	8.0×10^7	30.0×10^7

Appendix table 3: Survival and/or growth of *L. monocytogenes* in packaged beef (20) containing either 30%:70% CO_2:N_2 or air.

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
30%:70% CO_2:N_2 / 5	0	7.0×10^4	1.1×10^5	1.4×10^5
		1.0×10^5	1.3×10^5	9.0×10^4
		2.4×10^5	1.0×10^5	2.5×10^5
	7	70.0×10^4	30.0×10^4	20.0×10^4
		10.0×10^4	60.0×10^4	90.0×10^4
		20.0×10^4	70.0×10^4	50.0×10^4
	14	6.0×10^4	4.0×10^4	2.0×10^4
		6.0×10^4	8.0×10^4	5.0×10^4
		4.0×10^4	8.0×10^4	4.0×10^4
	21	40.0×10^4	50.0×10^4	40.0×10^4
		20.0×10^4	30.0×10^4	20.0×10^4
		50.0×10^4	40.0×10^4	40.0×10^4
	28	1.9×10^5	1.4×10^5	3.9×10^5
		1.9×10^5	30.0×10^4	3.6×10^5
		2.6×10^5	40.0×10^4	3.6×10^5
30%:70% CO_2:N_2 / 11	0	20.0×10^5	10.4×10^5	10.7×10^5
		10.1×10^5	10.0×10^5	20.2×10^5
		10.0×10^5	10.0×10^5	10.4×10^5
	7	5.0×10^5	10.0×10^5	20.0×10^5
		7.0×10^5	6.0×10^5	16.0×10^5
		7.0×10^5	45.0×10^5	22.0×10^5
	14	4.2×10^7	10.2×10^7	8.0×10^7
		4.1×10^7	80.0×10^7	8.2×10^7
		1.6×10^7	80.0×10^7	8.0×10^7
	21	70.0×10^7	7.0×10^8	70.0×10^7
		50.0×10^7	1.0×10^8	60.0×10^7
		70.0×10^7	1.4×10^8	60.0×10^7
Air / 5	0	2.3×10^5	3.0×10^5	70.0×10^4
		1.2×10^5	1.6×10^5	1.0×10^5
		90.0×10^4	1.7×10^5	2.4×10^5
	7	2.0×10^4	3.0×10^4	2.0×10^4
		2.0×10^4	2.0×10^4	2.0×10^4
		1.0×10^4	3.0×10^4	2.3×10^4
	14	12.0×10^4	70.0×10^4	70.0×10^4
		20.0×10^4	70.0×10^4	70.0×10^4
		15.0×10^4	70.0×10^4	60.0×10^4
	21	10.1×10^5	20.8×10^5	40.5×10^5
		10.1×10^5	20.3×10^5	60.2×10^5
		90.0×10^4	20.3×10^5	40.5×10^5
	28	3.2×10^6	2.2×10^6	2.4×10^6
		2.0×10^6	2.1×10^6	2.6×10^6
		2.3×10^6	2.4×10^6	2.5×10^6
Air / 11	0	90.0×10^4	1.5×10^5	2.1×10^5
		10.3×10^5	12.0×10^5	17.0×10^5

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
		20.2×10^5	10.5×10^5	10.6×10^5
	7	20.0×10^5	60.0×10^5	30.0×10^5
		30.0×10^5	30.0×10^5	30.0×10^5
		10.0×10^5	10.0×10^5	20.2×10^5
	14	10.3×10^7	30.0×10^7	30.9×10^7
		10.2×10^7	30.9×10^7	40.9×10^7
		10.2×10^7	30.3×10^7	20.7×10^7
	21	90.0×10^7	90.0×10^7	80.0×10^7
		90.0×10^7	4.0×10^8	70.0×10^7
		10.1×10^8	1.4×10^8	1.2×10^8

Appendix table 4: Survival and/or growth of *L. monocytogenes* in packaged beef (20) containing either 30%:70% CO_2:N_2 or air followed by irradiation at 1.75 kGy.

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
30%:70% CO_2:N_2 / 5	0	4.1×10^2	7.9×10^2	8.9×10^2
		4.3×10^2	6.4×10^2	4.4×10^2
		5.5×10^2	7.7×10^2	4.4×10^2
	7	1.0×10^2	9.0×10^1	2.1×10^2
		1.1×10^2	8.0×10^1	1.1×10^2
		1.7×10^2	1.8×10^2	2.4×10^2
	14	30.1×10^2	10.4×10^2	10.1×10^2
		20.9×10^2	10.2×10^2	10.5×10^2
		40.4×10^2	10.1×10^2	5.0×10^1
	21	6.6×10^2	60.0×10^2	30.3×10^2
		42.0×10^2	9.0×10^2	30.3×10^2
		7.1×10^2	40.0×10^2	30.3×10^2
	28	1.2×10^2	1.0×10^1	8.0×10^1
		6.0×10^1	2.0×10^1	7.0×10^1
		1.1×10^2	5.0×10^1	10.0×10^2
	35	27.0×10^3	3.6×10^3	1.1×10^3
		10.0×10^2	3.1×10^3	4.0×10^3
		12.0×10^2	1.5×10^3	7.3×10^3
	42	5.0×10^2	5.0×10^2	8.2×10^3
		3.0×10^2	5.0×10^2	6.9×10^3
		6.0×10^2	9.0×10^2	8.3×10^3
30%:70% CO_2:N_2 / 11	0	6.0×10^3	23.0×10^3	11.0×10^3
		9.0×10^3	13.0×10^3	3.0×10^3
		14.0×10^3	11.0×10^3	8.0×10^3
	7	15.0×10^2	5.0×10^2	90.0×10^1
		90.0×10^1	70.0×10^1	4.0×10^2
		11.0×10^2	2.0×10^2	70.0×10^1
	14	60.0×10^1	30.0×10^1	40.0×10^1
		40.0×10^1	60.0×10^1	40.1×10^1
		30.0×10^1	30.0×10^1	40.1×10^1
	21	1.2×10^4	1.7×10^4	1.1×10^4
		1.9×10^4	1.7×10^4	1.5×10^4
		1.7×10^4	1.5×10^4	1.5×10^4
	28	1.1×10^7	2.9×10^7	2.0×10^7
		1.1×10^7	90.1×10^6	90.2×10^6
		70.3×10^6	11.0×10^7	70.8×10^6
Air / 5	0	20.8×10^2	20.3×10^2	20.7×10^2
		20.7×10^2	20.0×10^2	40.2×10^2
		20.5×10^2	20.7×10^2	30.1×10^2
	7	20.0×10^1	30.0×10^1	11.0×10^1
		30.0×10^1	30.0×10^1	60.0×10^1
		60.0×10^1	50.0×10^1	70.0×10^1
	14	1.0×10^1	1.1×10^1	1.5×10^1
		3.0×10^1	1.5×10^1	1.6×10^1

Atmosphere/ Temperature (^{o}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
Air / 11	0	1.0×10^{1}	1.5×10^{1}	1.6×10^{1}
	21	6.0×10^{1}	7.0×10^{1}	18.0×10^{1}
		6.0×10^{1}	2.0×10^{1}	6.0×10^{1}
		11.0×10^{1}	5.0×10^{1}	5.0×10^{1}
	28	80.0×10^{3}	15.0×10^{4}	80.2×10^{3}
		20.0×10^{3}	15.0×10^{4}	80.3×10^{3}
		20.0×10^{3}	14.0×10^{4}	80.3×10^{3}
	35	1.7×10^{4}	1.6×10^{4}	2.0×10^{4}
		1.6×10^{4}	1.7×10^{4}	2.0×10^{4}
		1.9×10^{4}	2.2×10^{4}	2.2×10^{4}
	42	17.0×10^{5}	20.0×10^{5}	59.0×10^{5}
		20.0×10^{5}	40.0×10^{5}	80.0×10^{5}
		22.0×10^{5}	20.0×10^{5}	88.0×10^{5}
	0	10.0×10^{2}	10.0×10^{2}	20.0×10^{2}
		10.0×10^{2}	30.0×10^{2}	7.0×10^{2}
		30.0×10^{2}	10.0×10^{2}	24.0×10^{2}
	7	31.0×10^{4}	7.0×10^{4}	6.0×10^{4}
		30.0×10^{4}	1.3×10^{4}	9.0×10^{4}
		4.7×10^{4}	70.0×10^{4}	4.0×10^{4}
	14	1.1×10^{5}	1.9×10^{5}	1.5×10^{5}
		1.2×10^{5}	1.9×10^{5}	1.5×10^{5}
		98.0×10^{4}	2.1×10^{5}	1.5×10^{5}
	21	2.3×10^{6}	1.0×10^{6}	1.5×10^{6}
		1.6×10^{6}	1.1×10^{6}	1.5×10^{6}
		2.1×10^{6}	1.1×10^{6}	1.5×10^{6}
	28	7.0×10^{7}	10.0×10^{7}	10.0×10^{7}
		20.0×10^{7}	30.0×10^{7}	60.0×10^{7}
		8.0×10^{7}	8.0×10^{7}	25.0×10^{7}

Appendix table 5: Survival and/or growth of *L. monocytogenes* in packaged beef (47) containing either 15%:85% CO_2:N_2 or air.

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
15%:85% CO_2:N_2 / 5	0	18.0×10^5	23.0×10^5	23.0×10^5
		15.0×10^5	24.0×10^5	19.0×10^5
		19.0×10^5	25.0×10^5	20.0×10^5
	7	11.0×10^4	11.0×10^4	15.0×10^4
		19.0×10^4	80.0×10^4	16.0×10^4
		19.0×10^4	17.0×10^4	22.0×10^4
	14	50.0×10^4	55.0×10^4	20.0×10^4
		50.0×10^4	40.0×10^4	70.0×10^4
		80.0×10^4	70.0×10^4	85.0×10^4
	21	1.0×10^4	1.0×10^4	1.0×10^4
		3.0×10^4	1.0×10^4	9.0×10^4
		4.0×10^4	3.0×10^4	8.0×10^4
15%:85% CO_2:N_2 / 11	0	18.0×10^5	23.0×10^5	23.0×10^5
		15.0×10^5	24.0×10^5	19.0×10^5
		19.0×10^5	25.0×10^5	20.0×10^5
	7	10.0×10^6	10.0×10^6	61.0×10^6
		7.0×10^6	20.0×10^6	20.0×10^6
		6.0×10^6	25.0×10^6	50.0×10^6
	14	63.0×10^7	39.0×10^7	80.0×10^7
		61.0×10^7	4.0×10^8	66.0×10^7
		65.0×10^7	70.0×10^7	36.0×10^7
Air / 5	0	18.0×10^5	23.0×10^5	23.0×10^5
		15.0×10^5	24.0×10^5	19.0×10^5
		19.0×10^5	25.0×10^5	20.0×10^5
	7	18.0×10^4	17.0×10^4	11.0×10^4
		25.0×10^4	17.0×10^4	29.0×10^4
		19.0×10^4	19.0×10^4	29.0×10^4
	14	30.0×10^4	55.0×10^4	12.0×10^4
		44.0×10^4	70.0×10^4	17.0×10^4
		33.0×10^4	70.0×10^4	85.0×10^4
	21	28.0×10^4	11.0×10^4	20.0×10^4
		19.0×10^4	11.0×10^4	13.0×10^4
		20.0×10^4	19.0×10^4	12.0×10^4
Air / 11	0	18.0×10^5	23.0×10^5	23.0×10^5
		15.0×10^5	24.0×10^5	19.0×10^5
		19.0×10^5	25.0×10^5	20.0×10^5
	7	27.5×10^8	31.2×10^8	28.5×10^8
		23.4×10^8	27.5×10^8	26.5×10^8
		27.7×10^8	27.5×10^8	27.5×10^8
	14	11.0×10^8	40.0×10^8	38.0×10^8
		10.0×10^8	5.6×10^8	40.0×10^8
		6.8×10^8	5.7×10^8	4.7×10^8

Appendix table 6: Survival and/or growth of *L. monocytogenes* in packaged beef (47) containing either 15%:85% CO_2:N_2 or air followed by irradiation at 1.75 kGy.

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
15%:85% CO_2:N_2 / 5	0	50.0×10^2	30.0×10^2	70.0×10^2
		60.0×10^2	30.0×10^2	40.0×10^2
		40.0×10^2	10.0×10^2	53.0×10^2
	7	80.0×10^1	5.0×10^2	80.0×10^1
		14.0×10^2	4.0×10^2	11.0×10^2
		60.0×10^1	5.0×10^2	3.0×10^2
	14	30.0×10^1	20.0×10^1	40.0×10^1
		40.0×10^1	60.0×10^1	84.0×10^1
		40.0×10^1	80.0×10^1	84.0×10^1
	21	30.0×10^1	10.0×10^1	41.0×10^1
		10.0×10^1	10.0×10^1	42.0×10^1
		30.0×10^1	20.0×10^1	41.9×10^1
	28	1.0×10^2	2.0×10^2	1.0×10^2
		1.9×10^2	1.0×10^2	1.3×10^2
		2.3×10^2	1.0×10^2	1.3×10^2
	35	2.0×10^1	2.0×10^1	2.0×10^1
		2.0×10^1	1.0×10^1	2.0×10^1
		1.0×10^1	1.0×10^1	1.6×10^1
	42	10.0×10^1	10.0×10^1	12.0×10^1
		10.0×10^1	10.0×10^1	12.0×10^1
		20.0×10^1	10.0×10^1	80.4×10^1
15%:85% CO_2:N_2 / 11	0	50.0×10^2	30.0×10^2	70.0×10^2
		60.0×10^2	30.0×10^2	40.0×10^2
		40.0×10^2	10.0×10^2	53.0×10^2
	7	51.0×10^3	30.0×10^3	31.0×10^4
		46.0×10^3	70.0×10^3	37.0×10^4
		38.0×10^3	70.0×10^3	36.0×10^4
	14	22.0×10^7	1.1×10^7	24.0×10^7
		1.3×10^7	1.4×10^7	23.0×10^7
		1.3×10^7	1.4×10^7	20.0×10^7
	21	1.3×10^8	1.7×10^8	1.6×10^8
		1.2×10^8	1.5×10^8	37.2×10^8
		1.6×10^8	1.3×10^8	1.8×10^8
	28	1.0×10^8	70.0×10^7	1.1×10^8
		1.1×10^8	90.0×10^7	1.2×10^8
		1.5×10^8	1.7×10^8	1.6×10^8
Air / 5	0	10.0×10^2	30.0×10^2	70.0×10^2
		80.0×10^2	20.0×10^2	10.0×10^2
		50.0×10^2	50.0×10^2	30.0×10^2
	7	60.0×10^1	50.0×10^1	40.0×10^1
		20.0×10^1	20.0×10^1	80.0×10^1
		70.0×10^1	30.0×10^1	20.0×10^1
	14	50.0×10^1	70.0×10^1	10.0×10^1
		40.0×10^1	60.0×10^1	10.0×10^1

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
		50.0×10^1	10.0×10^1	36.0×10^1
	21	10.0×10^1	10.0×10^1	20.0×10^1
		10.0×10^1	40.0×10^1	20.0×10^1
		10.0×10^1	40.0×10^1	20.0×10^1
	28	2.5×10^2	2.9×10^2	2.7×10^2
		3.3×10^2	3.1×10^2	1.5×10^2
		3.6×10^2	3.2×10^2	3.8×10^2
	35	10.0×10^1	10.0×10^1	90.0×10^1
		10.0×10^1	30.0×10^1	90.6×10^1
		10.0×10^1	70.0×10^1	90.3×10^1
	42	10.0×10^1	50.0×10^1	10.0×10^1
		10.0×10^1	50.0×10^1	10.0×10^1
		10.0×10^1	40.0×10^1	10.0×10^1
Air /	0	10.0×10^2	30.0×10^2	70.0×10^2
11		80.0×10^2	20.0×10^2	10.0×10^2
		50.0×10^2	50.0×10^2	30.0×10^2
	7	50.0×10^4	60.0×10^4	60.0×10^4
		50.9×10^4	40.0×10^4	50.0×10^4
		40.0×10^4	58.0×10^4	60.0×10^4
	14	30.7×10^6	50.2×10^6	40.2×10^6
		30.3×10^6	60.3×10^6	40.9×10^6
		70.2×10^6	40.5×10^6	40.9×10^6
	21	72.0×10^8	39.0×10^8	45.0×10^8
		34.0×10^8	30.0×10^8	45.0×10^8
		52.0×10^8	42.0×10^8	45.0×10^8
	28	42.0×10^8	29.0×10^8	36.0×10^8
		30.0×10^8	42.0×10^8	37.0×10^8
		37.0×10^8	36.0×10^8	35.0×10^8

Appendix table 7: Survival and/or growth of *L. monocytogenes* in packaged beef (47) containing either 30%:70% CO_2:N_2 or air.

Atmosphere/ Temperature (°C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
30%:70% CO_2:N_2 / 5	0	18.0×10^5	17.0×10^5	15.0×10^5
		16.0×10^5	17.0×10^5	16.0×10^5
		16.0×10^5	13.0×10^5	12.0×10^5
	7	40.0×10^4	45.0×10^4	47.0×10^4
		49.0×10^4	45.0×10^4	44.0×10^4
		49.0×10^4	46.0×10^4	47.0×10^4
	14	1.0×10^4	1.0×10^4	12.0×10^4
		6.0×10^4	3.0×10^4	4.0×10^4
		13.0×10^4	7.0×10^4	8.0×10^4
	21	30.0×10^4	30.0×10^4	30.0×10^4
		32.0×10^4	30.0×10^4	31.0×10^4
		35.0×10^4	30.0×10^4	35.0×10^4
	28	43.0×10^4	40.0×10^4	42.0×10^4
		46.0×10^4	41.0×10^4	41.0×10^4
		40.0×10^4	46.0×10^4	43.0×10^4
30%:70% CO_2:N_2 / 11	0	24.0×10^5	24.0×10^5	26.0×10^5
		18.0×10^5	25.0×10^5	32.0×10^5
		20.0×10^5	30.0×10^5	41.0×10^5
	7	51.0×10^6	49.0×10^6	51.0×10^6
		58.0×10^6	52.0×10^6	51.0×10^6
		57.0×10^6	51.0×10^6	56.0×10^6
	14	6.7×10^8	8.2×10^8	94.0×10^7
		6.4×10^8	7.8×10^8	92.0×10^7
		5.9×10^8	6.5×10^8	94.0×10^7
	21	6.0×10^8	10.0×10^8	27.0×10^8
		8.0×10^8	4.0×10^8	28.0×10^8
		12.0×10^8	2.4×10^8	27.0×10^8
Air / 5	0	24.0×10^5	24.0×10^5	26.0×10^5
		18.0×10^5	25.0×10^5	32.0×10^5
		20.0×10^5	30.0×10^5	41.0×10^5
	7	26.0×10^5	16.0×10^5	26.0×10^5
		28.0×10^5	25.0×10^5	30.0×10^5
		29.0×10^5	24.0×10^5	30.5×10^5
	14	13.0×10^5	44.0×10^5	85.0×10^5
		18.0×10^5	55.0×10^5	35.0×10^5
		18.0×10^5	54.0×10^5	76.0×10^5
	21	1.1×10^6	60.0×10^5	1.0×10^6
		1.1×10^6	5.6×10^6	1.1×10^6
		1.1×10^6	4.2×10^6	2.1×10^6
	28	3.0×10^6	54.0×10^6	5.0×10^6
		6.0×10^6	61.0×10^6	5.0×10^6
		4.0×10^6	68.0×10^6	5.0×10^6
Air / 11	0	24.0×10^5	24.0×10^5	26.0×10^5
		18.0×10^5	25.0×10^5	32.0×10^5
		20.0×10^5	30.0×10^5	41.0×10^5

Atmosphere/ Temperature (^{o}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
	7	44.0×10^{6}	58.0×10^{6}	48.0×10^{6}
		88.0×10^{6}	59.0×10^{6}	37.0×10^{6}
		70.0×10^{6}	52.0×10^{6}	48.0×10^{6}
	14	7.4×10^{8}	15.1×10^{8}	5.0×10^{8}
		10.1×10^{8}	13.1×10^{8}	3.7×10^{8}
		6.7×10^{8}	17.3×10^{8}	3.8×10^{8}
	21	25.0×10^{8}	14.0×10^{8}	58.0×10^{8}
		17.0×10^{8}	16.0×10^{8}	15.0×10^{8}
		22.0×10^{8}	27.0×10^{8}	15.0×10^{8}

Appendix table 8: Survival and/or growth of *L. monocytogenes* in packaged beef (47) containing either 30%:70% CO_2:N_2 or air followed by irradiation at 1.75 kGy.

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
30%:70% CO_2:N_2 / 5	0	41.0×10^2	79.0×10^2	89.0×10^2
		43.0×10^2	64.0×10^2	76.0×10^2
		55.0×10^2	77.0×10^2	74.0×10^2
	7	1.3×10^2	5.0×10^2	20.0×10^2
		2.8×10^2	3.0×10^2	1.4×10^2
		7.0×10^2	2.0×10^2	1.4×10^2
	14	16.0×10^2	22.0×10^2	17.0×10^2
		19.0×10^2	16.0×10^2	24.0×10^2
		23.0×10^2	16.0×10^2	17.0×10^2
	21	1.6×10^2	5.0×10^2	2.6×10^2
		1.9×10^2	7.0×10^2	1.3×10^2
		7.0×10^2	8.0×10^2	1.6×10^2
	28	1.2×10^2	1.2×10^2	9.0×10^2
		7.0×10^2	6.0×10^2	7.0×10^2
		1.2×10^2	1.1×10^2	1.1×10^2
	35	1.2×10^2	3.0×10^2	70.0×10^1
		90.0×10^1	1.0×10^2	90.0×10^1
		1.5×10^2	1.0×10^2	1.0×10^2
	42	57.0×10^2	10.0×10^2	24.0×10^2
		51.0×10^2	30.0×10^2	24.0×10^2
		31.0×10^2	20.0×10^2	24.0×10^2
30%:70% CO_2:N_2 / 11	0	6.0×10^3	2.3×10^3	1.1×10^3
		90.0×10^2	1.3×10^3	3.0×10^3
		1.4×10^3	1.1×10^3	80.0×10^2
	7	8.0×10^2	1.8×10^2	1.3×10^2
		20.0×10^2	1.6×10^2	1.8×10^2
		1.9×10^2	1.7×10^2	8.0×10^2
	14	4.0×10^2	6.0×10^2	1.8×10^2
		6.0×10^2	80.0×10^1	2.2×10^2
		3.0×10^2	6.0×10^2	1.5×10^2
	21	1.8×10^4	1.9×10^4	2.2×10^4
		2.3×10^4	1.7×10^4	8.1×10^4
		8.2×10^4	2.4×10^4	41.0×10^4
	28	2.1×10^6	10.4×10^6	41.1×10^7
		1.7×10^6	9.7×10^6	60.0×10^6
		2.1×10^6	10.3×10^6	60.0×10^6
Air / 5	0	27.0×10^2	17.0×10^2	29.0×10^2
		34.0×10^2	28.0×10^2	29.0×10^2
		34.0×10^2	26.0×10^2	39.0×10^2
	7	47.0×10^2	24.0×10^2	77.0×10^2
		49.0×10^2	15.0×10^2	58.0×10^2
		40.0×10^2	81.0×10^2	49.0×10^2
	14	25.0×10^2	20.0×10^2	50.0×10^2

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
		18.0×10^2	24.0×10^2	11.0×10^2
		19.0×10^2	17.0×10^2	17.0×10^2
	21	27.0×10^2	6.5×10^2	25.0×10^2
		3.6×10^2	4.6×10^2	3.6×10^2
		3.5×10^2	4.3×10^2	4.7×10^2
	28	12.0×10^2	2.0×10^2	5.0×10^2
		15.0×10^2	8.0×10^2	11.0×10^2
		11.0×10^2	6.0×10^2	6.0×10^2
	35	8.0×10^2	2.9×10^2	5.0×10^2
		4.0×10^2	1.0×10^2	5.0×10^2
		7.0×10^2	2.0×10^2	9.0×10^2
	42	8.0×10^4	18.0×10^4	12.0×10^4
		12.0×10^4	9.0×10^4	13.0×10^4
		8.0×10^4	2.0×10^4	12.0×10^4
Air / 11	0	10.0×10^2	30.0×10^2	70.0×10^2
		80.0×10^2	20.0×10^2	10.0×10^2
		50.0×10^2	50.0×10^2	30.0×10^2
	7	50.0×10^2	60.0×10^2	6.0×10^3
		59.0×10^2	40.0×10^2	5.0×10^3
		40.0×10^2	58.0×10^2	6.0×10^3
	14	37.0×10^6	52.0×10^6	42.0×10^6
		33.0×10^6	63.0×10^6	10.0×10^7
		72.0×10^6	45.0×10^6	16.0×10^7
	21	42.0×10^8	20.9×10^9	36.0×10^8
		30.0×10^8	42.0×10^8	36.0×10^8
		37.0×10^8	36.0×10^8	37.0×10^8

Appendix table 9: Survival and/or growth of *L. monocytogenes* in packaged beef (50) containing either 15%:85% CO_2:N_2 or air.

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
15%:85% CO_2:N_2 / 5	0	16.0×10^5	8.0×10^5	14.0×10^5
		3.0×10^5	27.0×10^5	18.0×10^5
		27.0×10^5	25.0×10^5	21.0×10^5
	7	11.0×10^5	11.0×10^5	15.0×10^5
		1.9×10^5	6.0×10^5	16.0×10^5
		1.9×10^5	17.0×10^5	2.2×10^5
	14	8.0×10^5	10.0×10^5	40.0×10^5
		6.8×10^5	30.0×10^5	40.0×10^5
		7.0×10^5	10.0×10^5	40.0×10^5
	21	7.0×10^5	4.1×10^5	30.0×10^5
		10.0×10^5	6.4×10^5	20.0×10^5
		5.0×10^5	5.3×10^5	24.0×10^5
15%:85% CO_2:N_2 / 11	0	16.0×10^5	8.0×10^5	14.0×10^5
		3.0×10^5	27.0×10^5	18.0×10^5
		27.0×10^5	25.0×10^5	21.0×10^5
	7	19.0×10^8	30.4×10^8	26.4×10^8
		24.0×10^8	28.5×10^8	26.5×10^8
		27.0×10^8	26.4×10^8	26.5×10^8
	14	27.0×10^8	19.0×10^8	22.0×10^8
		17.0×10^8	23.0×10^8	17.0×10^8
		18.0×10^8	27.0×10^8	13.0×10^8
Air / 5	0	18.0×10^5	23.0×10^5	23.0×10^5
		15.0×10^5	24.0×10^5	19.0×10^5
		19.0×10^5	25.0×10^5	20.0×10^5
	7	18.0×10^5	17.0×10^5	11.0×10^5
		2.5×10^5	17.0×10^5	7.0×10^5
		19.0×10^5	9.0×10^5	20.0×10^5
	14	2.7×10^5	6.0×10^5	12.0×10^5
		2.5×10^5	4.0×10^5	17.0×10^5
		33.0×10^5	7.0×10^5	11.0×10^5
	21	3.0×10^7	31.0×10^7	16.0×10^7
		1.8×10^7	32.0×10^7	16.0×10^7
		2.3×10^7	26.0×10^7	16.0×10^7
Air / 11	0	18.0×10^5	23.0×10^5	23.0×10^5
		15.0×10^5	24.0×10^5	19.0×10^5
		19.0×10^5	25.0×10^5	20.0×10^5
	7	27.5×10^8	26.5×10^8	23.5×10^8
		23.4×10^8	28.5×10^8	28.7×10^8
		27.7×10^8	23.3×10^8	26.7×10^8
	14	31.0×10^8	19.0×10^8	49.0×10^8
		31.0×10^8	34.0×10^8	61.0×10^8
		39.0×10^8	24.0×10^8	31.0×10^8

Appendix table 10: Survival and/or growth of *L. monocytogenes* in packaged beef (50) containing either 15%:85% CO_2:N_2 or air followed by irradiation at 1.75 kGy.

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
15%:85% CO_2:N_2 / 5	0	20.0×10^2	70.0×10^2	40.0×10^2
		40.0×10^2	13.0×10^2	60.0×10^2
		60.0×10^2	80.0×10^2	20.0×10^2
	7	13.0×10^2	8.0×10^2	10.0×10^2
		5.0×10^2	14.0×10^2	95.0×10^1
		10.0×10^2	90.0×10^1	10.0×10^2
	14	11.0×10^2	50.0×10^2	6.0×10^2
		11.0×10^2	14.0×10^2	14.0×10^2
		15.0×10^2	8.0×10^2	16.0×10^2
	21	8.0×10^2	1.0×10^2	8.0×10^2
		16.0×10^2	1.0×10^2	1.0×10^2
		11.0×10^2	3.0×10^2	2.0×10^2
	28	4.0×10^2	70.0×10^1	3.0×10^2
		3.0×10^2	4.0×10^2	80.0×10^1
		12.0×10^2	4.0×10^2	50.0×10^1
	35	10.0×10^3	36.0×10^3	50.0×10^3
		20.0×10^3	31.0×10^3	97.0×10^2
		20.0×10^3	50.0×10^3	56.0×10^3
	42	40.0×10^1	40.0×10^1	30.0×10^1
		40.0×10^1	40.0×10^1	60.0×10^1
		40.0×10^1	40.0×10^1	70.0×10^1
15%:85% CO_2:N_2 / 11	0	20.0×10^2	70.0×10^2	40.0×10^2
		40.0×10^2	13.0×10^2	60.0×10^2
		60.0×10^2	80.0×10^2	20.0×10^2
	7	29.0×10^3	14.0×10^3	6.0×10^3
		12.0×10^3	17.0×10^3	26.0×10^3
		38.0×10^3	19.0×10^3	19.0×10^3
	14	45.0×10^6	39.0×10^6	67.0×10^6
		72.0×10^6	87.0×10^6	66.0×10^6
		63.0×10^6	94.0×10^6	67.0×10^6
	21	16.0×10^8	60.0×10^7	5.0×10^8
		60.0×10^7	13.0×10^8	80.0×10^7
		2.1×10^8	5.0×10^8	12.0×10^8
	28	9.0×10^8	24.0×10^8	26.0×10^8
		17.0×10^8	22.0×10^8	30.0×10^8
		12.0×10^8	18.0×10^8	23.0×10^8
Air / 5	0	50.0×10^2	30.0×10^2	75.0×10^2
		40.0×10^2	10.0×10^2	55.0×10^2
		80.0×10^2	30.0×10^2	65.0×10^2
	7	10.0×10^2	6.0×10^2	4.5×10^2
		14.0×10^2	6.0×10^2	14.0×10^2
		9.0×10^2	7.0×10^2	14.0×10^2
	14	8.0×10^2	3.0×10^2	6.5×10^2
		4.0×10^2	8.0×10^2	7.0×10^2

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
		8.0×10^2	8.0×10^2	7.0×10^2
	21	80.0×10^1	1.0×10^2	80.0×10^1
		16.0×10^2	1.0×10^2	1.0×10^2
		11.0×10^2	3.0×10^2	2.0×10^2
	28	10.0×10^1	11.0×10^1	40.0×10^1
		30.0×10^1	60.0×10^1	40.0×10^1
		20.0×10^1	30.0×10^1	45.0×10^1
	35	20.0×10^3	90.0×10^3	16.1×10^3
		20.0×10^3	90.0×10^3	8.6×10^3
		60.0×10^3	14.0×10^3	18.5×10^3
	42	40.0×10^1	40.0×10^1	30.0×10^1
		60.0×10^1	40.0×10^1	60.0×10^1
		60.0×10^1	38.0×10^1	90.0×10^1
Air / 11	0	50.0×10^2	30.0×10^2	75.0×10^2
		40.0×10^2	10.0×10^2	55.0×10^2
		80.0×10^2	30.0×10^2	65.0×10^2
	7	32.0×10^3	48.0×10^3	10.7×10^4
		30.0×10^3	40.0×10^3	10.8×10^4
		42.0×10^3	46.0×10^3	10.9×10^4
	14	92.0×10^6	75.0×10^6	84.0×10^6
		72.0×10^6	97.0×10^6	85.0×10^6
		88.0×10^6	82.0×10^6	84.0×10^6
	21	2.0×10^7	28.0×10^7	14.0×10^7
		4.0×10^7	18.0×10^7	25.0×10^7
		2.0×10^7	14.0×10^7	33.0×10^7
	28	15.0×10^8	32.0×10^8	22.0×10^8
		28.0×10^8	21.0×10^8	17.0×10^8
		34.0×10^8	27.0×10^8	12.0×10^8

Appendix table 11: Survival and/or growth of *L. monocytogenes* in packaged beef (50) containing either 30%:70% CO_2:N_2 or air.

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
30%:85% CO_2:N_2 / 5	0	10.4×10^5	13.0×10^5	9.0×10^5
		15.0×10^5	24.0×10^5	14.0×10^5
		22.0×10^5	15.0×10^5	26.0×10^5
	7	13.0×10^5	10.0×10^5	70.0×10^4
		90.0×10^4	5.0×10^5	84.0×10^4
		11.0×10^5	4.0×10^5	84.0×10^4
	14	40.0×10^4	50.0×10^4	30.0×10^4
		40.0×10^4	60.0×10^4	40.0×10^4
		30.0×10^4	60.0×10^4	60.0×10^4
	21	20.0×10^4	10.0×10^4	30.0×10^4
		10.0×10^4	10.0×10^4	20.0×10^4
		30.0×10^4	20.0×10^4	30.0×10^4
	28	20.0×10^4	40.0×10^4	20.0×10^4
		20.0×10^4	20.0×10^4	30.0×10^4
		20.0×10^4	20.0×10^4	20.0×10^4
30%:85% CO_2:N_2 / 11	0	10.4×10^5	13.0×10^5	9.0×10^5
		15.0×10^5	24.0×10^5	14.0×10^5
		22.0×10^5	15.0×10^5	26.0×10^5
	7	96.0×10^6	4.6×10^7	13.0×10^7
		11.6×10^7	6.9×10^7	16.0×10^7
		11.0×10^7	6.6×10^7	17.0×10^7
	14	13.0×10^8	11.0×10^8	12.0×10^8
		80.0×10^7	12.0×10^8	10.0×10^8
		11.0×10^8	11.0×10^8	11.0×10^8
Air / 5	0	18.0×10^5	21.0×10^5	10.9×10^5
		21.0×10^5	60.0×10^5	15.0×10^5
		22.0×10^5	23.0×10^5	15.0×10^5
	7	15.0×10^5	15.0×10^5	7.0×10^5
		16.0×10^5	16.0×10^5	8.0×10^5
		26.0×10^5	16.0×10^5	8.0×10^5
	14	19.0×10^5	27.0×10^5	36.0×10^5
		21.0×10^5	37.0×10^5	20.0×10^5
		22.0×10^5	26.0×10^5	55.0×10^5
	21	12.2×10^5	40.0×10^5	40.0×10^5
		10.8×10^5	57.0×10^5	20.0×10^5
		12.0×10^5	57.0×10^5	46.0×10^5
	28	10.0×10^5	17.0×10^5	50.0×10^5
		30.0×10^5	30.0×10^5	36.0×10^5
		20.0×10^5	40.0×10^5	35.0×10^5
Air / 11	0	18.0×10^5	21.0×10^5	10.9×10^5
		21.0×10^5	60.0×10^5	15.0×10^5
		22.0×10^5	23.0×10^5	15.0×10^5
	7	7.3×10^8	96.0×10^7	25.0×10^8
		6.9×10^8	12.4×10^8	11.5×10^8
		1.1×10^8	11.2×10^8	80.0×10^7

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
	14	22.0×10^8	23.0×10^8	10.0×10^8
		29.0×10^8	25.0×10^8	14.0×10^8
		22.0×10^8	35.0×10^8	13.0×10^8

Appendix table 12: Survival and/or growth of *L. monocytogenes* in packaged beef (50) containing either 30%:70% CO_2:N_2 or air followed by irradiation at 1.75 kGy.

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
30%:70% CO_2:N_2 / 5	0	92.0×10^2	83.0×10^2	53.0×10^2
		92.0×10^2	98.0×10^2	57.0×10^2
		88.0×10^2	45.0×10^2	76.0×10^2
	7	1.0×10^1	1.0×10^1	79.0×10^0
		1.0×10^1	1.0×10^1	68.0×10^0
		1.0×10^1	1.0×10^1	98.0×10^0
	14	49.0×10^2	21.0×10^2	15.0×10^2
		38.0×10^2	25.0×10^2	16.0×10^2
		29.0×10^2	31.0×10^2	11.0×10^2
	21	28.0×10^2	12.0×10^2	4.0×10^2
		22.0×10^2	10.0×10^2	5.0×10^2
		26.0×10^2	10.0×10^2	7.0×10^2
	28	5.0×10^2	6.0×10^1	21.0×10^2
		42.0×10^2	14.0×10^2	16.0×10^2
		5.1×10^2	6.0×10^1	19.0×10^2
	35	4.0×10^2	6.0×10^2	12.0×10^2
		5.0×10^2	90.0×10^1	17.0×10^2
		4.0×10^2	3.0×10^2	29.0×10^2
	42	20.0×10^2	10.0×10^2	21.0×10^2
		29.0×10^2	12.0×10^2	21.0×10^2
		34.0×10^2	21.0×10^2	21.0×10^2
30%:70% CO_2:N_2 / 11	0	39.0×10^2	10.0×10^2	24.0×10^2
		34.0×10^2	12.0×10^2	37.0×10^2
		31.0×10^2	14.0×10^2	45.0×10^2
	7	8.0×10^2	43.0×10^2	3.0×10^2
		3.0×10^2	52.0×10^2	5.0×10^2
		3.0×10^2	47.0×10^2	5.0×10^2
	14	20.0×10^3	93.1×10^3	62.0×10^3
		26.0×10^3	91.0×10^3	63.0×10^3
		23.0×10^3	92.9×10^3	63.0×10^3
	21	17.5×10^6	18.5×10^6	20.9×10^6
		20.8×10^6	16.5×10^6	29.2×10^6
		28.2×10^6	20.7×10^6	27.2×10^6
	28	25.0×10^7	12.0×10^7	18.0×10^7
		31.0×10^7	7.0×10^7	18.0×10^7
		28.0×10^7	7.0×10^7	18.5×10^7
Air / 5	0	11.0×10^2	23.0×10^2	41.0×10^2
		24.0×10^2	20.0×10^2	42.0×10^2
		16.0×10^2	26.0×10^2	30.0×10^2
	7	1.0×10^1	1.0×10^1	1.0×10^1
		2.0×10^1	1.0×10^1	1.0×10^1
		1.0×10^1	2.0×10^1	1.3×10^1
	14	90.0×10^1	3.8×10^2	13.0×10^2
		8.0×10^2	2.4×10^2	14.0×10^2

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
		8.0×10^2	26.0×10^2	12.0×10^2
	21	1.5×10^3	90.0×10^2	1.8×10^3
		2.4×10^3	90.0×10^2	1.4×10^3
		3.3×10^3	89.0×10^2	5.0×10^3
	28	63.0×10^3	63.0×10^3	97.0×10^3
		67.0×10^3	67.0×10^3	97.0×10^3
		71.0×10^3	71.0×10^3	62.0×10^3
	35	1.3×10^4	1.3×10^4	8.0×10^4
		2.8×10^4	1.8×10^4	1.0×10^4
		3.9×10^4	1.2×10^4	7.0×10^4
	42	2.6×10^5	10.0×10^5	3.0×10^5
		21.0×10^5	8.0×10^5	3.0×10^5
		3.3×10^5	7.0×10^5	13.0×10^5
Air / 11	0	19.0×10^2	20.0×10^2	29.0×10^2
		13.0×10^2	26.0×10^2	15.0×10^2
		26.0×10^2	29.0×10^2	22.0×10^2
	7	68.0×10^2	33.0×10^2	80.0×10^2
		64.0×10^2	23.0×10^2	62.0×10^2
		47.0×10^2	28.0×10^2	57.0×10^2
	14	29.4×10^6	30.4×10^6	28.8×10^6
		28.7×10^6	28.4×10^6	26.3×10^6
		26.4×10^6	28.6×10^6	26.5×10^6
	21	7.0×10^7	45.0×10^7	44.0×10^7
		8.0×10^7	51.0×10^7	46.0×10^7
		2.0×10^7	33.0×10^7	40.0×10^7
	28	10.0×10^7	30.0×10^7	40.0×10^7
		10.0×10^7	80.0×10^7	70.0×10^7
		10.0×10^7	70.0×10^7	50.0×10^7

Appendix table 13: Survival and/or growth of *L. monocytogenes* in packaged beef (54) containing either 15%:85% CO_2:N_2 or air.

Atmosphere/ Temperature (°C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
15%:85% CO_2:N_2 / 5	0	94.0×10^5	97.0×10^5	55.0×10^5
		10.7×10^5	85.0×10^5	65.0×10^5
		92.0×10^5	67.0×10^5	83.0×10^5
	7	54.0×10^5	38.0×10^5	73.0×10^5
		65.0×10^5	32.0×10^5	57.0×10^5
		69.0×10^5	46.0×10^5	36.0×10^5
	14	20.0×10^5	20.0×10^5	30.0×10^5
		30.0×10^5	20.0×10^5	30.0×10^5
		50.0×10^5	40.0×10^5	30.0×10^5
	21	90.0×10^5	30.0×10^5	30.0×10^5
		40.0×10^5	10.0×10^5	60.0×10^5
		50.0×10^5	40.0×10^5	40.0×10^5
15%:85% CO_2:N_2 / 11	0	94.0×10^5	97.0×10^5	55.0×10^5
		10.7×10^5	85.0×10^5	65.0×10^5
		92.0×10^5	67.0×10^5	83.0×10^5
	7	16.0×10^7	15.0×10^7	2.0×10^7
		3.0×10^7	60.0×10^6	4.0×10^7
		2.0×10^7	60.0×10^6	70.0×10^6
	14	21.0×10^8	8.0×10^8	24.0×10^8
		24.0×10^8	16.0×10^8	32.0×10^8
		16.0×10^8	14.0×10^8	24.0×10^8
Air / 5	0	94.0×10^5	92.0×10^5	77.0×10^5
		97.0×10^5	97.0×10^5	23.0×10^5
		20.0×10^5	85.0×10^5	19.0×10^5
	7	44.0×10^5	53.0×10^5	60.0×10^5
		63.0×10^5	70.0×10^5	68.0×10^5
		42.0×10^5	76.0×10^5	82.0×10^5
	14	30.0×10^5	40.0×10^5	70.0×10^5
		40.0×10^5	30.0×10^5	40.0×10^5
		50.0×10^5	80.0×10^5	60.0×10^5
	21	9.0×10^6	30.0×10^6	6.0×10^6
		9.0×10^6	6.0×10^6	7.0×10^6
		6.0×10^6	5.0×10^6	6.0×10^6
Air / 11	0	94.0×10^5	92.0×10^5	77.0×10^5
		97.0×10^5	97.0×10^5	23.0×10^5
		20.0×10^5	85.0×10^5	19.0×10^5
	7	9.0×10^6	2.0×10^6	58.0×10^6
		3.0×10^6	1.0×10^6	47.0×10^6
		9.0×10^6	4.7×10^6	47.0×10^6
	14	96.0×10^6	21.1×10^6	96.0×10^6
		68.0×10^6	14.5×10^6	96.0×10^6
		94.0×10^6	17.8×10^6	96.0×10^6

Appendix table 14: Survival and/or growth of *L. monocytogenes* in packaged beef (54) containing either 15%:85% CO_2:N_2 or air followed by irradiation at 1.75 kGy.

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
15%:85% CO_2:N_2 / 5	0	34.0×10^3	90.0×10^3	66.0×10^3
		43.0×10^3	84.0×10^3	64.0×10^3
		75.0×10^3	69.0×10^3	65.0×10^3
	7	78.0×10^3	84.0×10^3	7.1×10^3
		97.0×10^3	80.0×10^3	8.1×10^3
		98.0×10^3	58.0×10^3	8.1×10^3
	14	19.0×10^3	18.0×10^3	40.0×10^3
		24.0×10^3	18.0×10^3	57.0×10^3
		45.0×10^3	27.0×10^3	54.0×10^3
	21	16.0×10^2	38.0×10^3	12.0×00^3
		11.0×10^3	18.0×10^3	7.0×10^3
		8.0×10^3	12.0×10^3	8.0×10^3
	28	7.0×10^3	23.0×10^3	21.0×10^3
		7.0×10^3	21.0×10^3	21.0×10^3
		11.0×10^3	17.0×10^3	16.0×10^3
	35	4.0×10^3	9.0×10^3	12.0×10^3
		12.0×10^3	10.0×10^3	9.0×10^3
		16.0×10^3	11.0×10^3	10.0×10^3
	42	20.0×10^2	10.0×10^2	41.0×10^2
		29.0×10^2	12.0×10^2	41.0×10^2
		34.0×10^2	21.0×10^2	41.0×10^2
15%:85% CO_2:N_2 / 11	0	3.4×10^3	90.0×10^2	6.4×10^3
		4.3×10^3	8.4×10^3	6.4×10^3
		7.5×10^3	6.9×10^3	6.4×10^3
	7	42.0×10^3	28.0×10^3	33.0×10^3
		42.0×10^3	32.0×10^3	36.0×10^3
		52.0×10^3	37.0×10^3	54.0×10^3
	14	18.0×10^7	34.0×10^7	12.0×10^7
		23.0×10^7	20.0×10^7	21.0×10^7
		51.0×10^7	47.0×10^7	19.0×10^7
	21	20.0×10^8	10.0×10^8	57.0×10^8
		57.0×10^8	13.0×10^8	66.0×10^8
		12.0×10^8	13.0×10^8	57.0×10^8
	28	8.0×10^7	9.0×10^7	39.0×10^7
		5.0×10^7	9.0×10^7	9.0×10^7
		7.0×10^7	10.0×10^7	7.0×10^7
Air / 5	0	34.0×10^3	77.0×10^3	15.0×10^3
		22.0×10^3	57.0×10^3	21.0×10^3
		30.0×10^3	26.0×10^3	24.0×10^3
	7	16.0×10^2	26.0×10^2	25.0×10^2
		22.0×10^2	20.0×10^2	18.0×10^2
		32.0×10^2	33.0×10^2	24.0×10^2
	14	7.0×10^1	10.0×10^1	16.0×10^1

Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
		15.0×10^2	12.0×10^2	12.0×10^2
		15.0×10^2	8.0×10^2	28.0×10^2
	21	80.0×10^1	20.0×10^1	30.0×10^1
		30.0×10^1	30.0×10^1	40.0×10^1
		20.0×10^1	50.0×10^1	60.0×10^1
	28	30.0×10^1	10.0×10^1	80.0×10^1
		40.0×10^1	30.0×10^1	80.0×10^1
		50.0×10^1	40.0×10^1	50.0×10^1
	35	20.0×10^1	10.0×10^1	10.0×10^1
		40.0×10^1	10.0×10^1	70.0×10^1
		60.0×10^1	30.0×10^1	20.0×10^1
	42	2.0×10^5	9.0×10^5	7.0×10^5
		8.0×10^5	4.0×10^5	20.0×10^5
		9.0×10^5	8.0×10^5	6.0×10^5
Air / 11	0	34.0×10^3	77.0×10^3	15.0×10^3
		22.0×10^3	57.0×10^3	21.0×10^3
		30.0×10^3	26.0×10^3	24.0×10^3
	7	1.0×10^3	1.0×10^3	13.0×10^3
		1.0×10^3	60.0×10^2	13.0×10^3
		2.0×10^3	50.0×10^2	13.0×10^3
	14	52.0×10^5	22.0×10^5	27.0×10^5
		88.0×10^5	72.0×10^5	54.0×10^5
		92.0×10^5	60.0×10^5	89.0×10^5
	21	13.0×10^8	17.0×10^8	13.5×10^8
		14.0×10^8	9.0×10^8	15.5×10^8
		15.0×10^8	13.0×10^8	14.5×10^8
	28	2.1×10^8	8.0×10^8	20.0×10^8
		3.0×10^8	12.0×10^8	20.0×10^8
		3.4×10^8	14.0×10^8	19.5×10^8

Appendix table 15: Survival and/or growth of *L. monocytogenes* in packaged beef (54) containing either 30%:70% CO_2:N_2 or air.

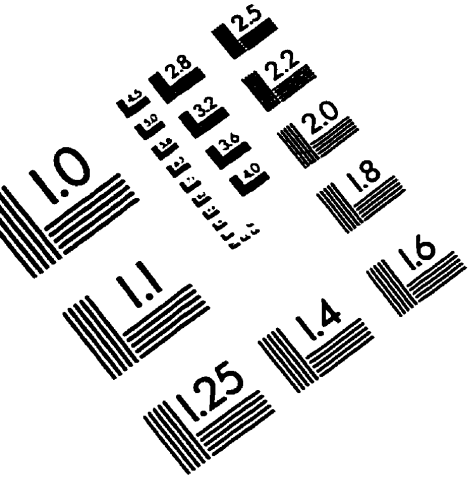
Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
30%:70% CO_2:N_2 / 5	0	10.6×10^5	12.4×10^5	56.0×10^4
		73.0×10^4	71.0×10^4	99.0×10^4
		95.0×10^4	12.2×10^5	84.0×10^4
	7	10.0×10^5	13.0×10^5	2.0×10^5
		10.0×10^5	10.0×10^5	3.0×10^5
		20.0×10^5	22.0×10^5	1.0×10^5
	14	23.0×10^5	9.0×10^5	12.0×10^5
		20.0×10^5	20.0×10^5	7.0×10^5
		13.0×10^5	14.0×10^5	11.0×10^5
	21	10.0×10^5	10.0×10^5	13.0×10^5
		10.0×10^5	20.0×10^5	13.0×10^5
		10.0×10^5	20.0×10^5	13.0×10^5
30%:70% CO_2:N_2 / 11	0	10.6×10^5	12.4×10^5	56.0×10^4
		73.0×10^4	71.0×10^4	99.0×10^4
		95.0×10^4	12.2×10^5	84.0×10^4
	7	20.0×10^5	40.0×10^5	13.0×10^5
		30.0×10^5	30.0×10^5	14.0×10^5
		40.0×10^5	40.0×10^5	10.0×10^5
	14	18.0×10^7	3.0×10^7	21.0×10^7
		19.0×10^7	3.0×10^7	47.0×10^7
		29.0×10^7	5.0×10^7	27.0×10^7
Air / 5	0	94.0×10^5	92.0×10^5	77.0×10^5
		97.0×10^5	97.0×10^5	23.0×10^5
		20.0×10^5	85.0×10^5	19.0×10^5
	7	44.0×10^5	53.0×10^5	60.0×10^5
		63.0×10^5	70.0×10^5	68.0×10^5
		42.0×10^5	76.0×10^5	82.0×10^5
	14	30.0×10^5	40.0×10^5	70.0×10^5
		40.0×10^5	30.0×10^5	40.0×10^5
		50.0×10^5	80.0×10^5	60.0×10^5
	21	9.0×10^6	30.0×10^6	6.0×10^6
		9.0×10^6	6.0×10^6	7.0×10^6
		6.0×10^6	5.0×10^6	6.0×10^6
Air / 11	0	94.0×10^5	92.0×10^5	77.0×10^5
		97.0×10^5	97.0×10^5	23.0×10^5
		20.0×10^5	85.0×10^5	19.0×10^5
	7	9.0×10^6	2.0×10^6	48.0×10^6
		3.0×10^6	1.0×10^6	47.0×10^6
		9.0×10^6	64.0×10^6	47.0×10^6
	14	96.0×10^7	51.1×10^7	13.0×10^8
		68.0×10^7	54.5×10^7	13.2×10^8
		94.0×10^7	57.8×10^7	13.4×10^8

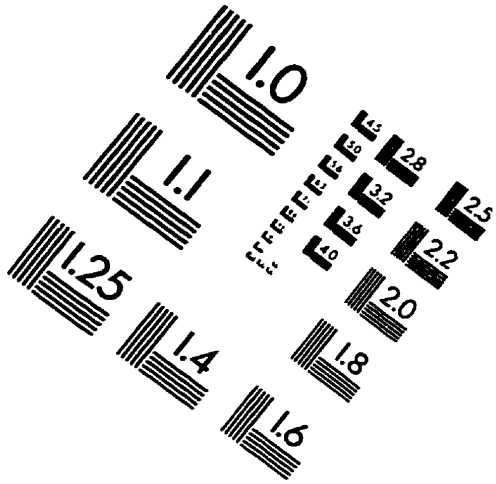
Appendix table 16: Survival and/or growth of *L. monocytogenes* in packaged beef (54) containing either 30%:70% CO_2:N_2 or air followed by irradiation at 1.75 kGy.

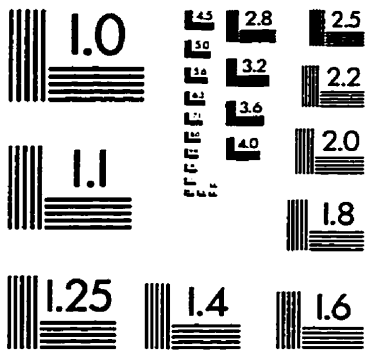
Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
30%:85% CO_2:N_2 / 5	0	34.0×10^3	77.0×10^3	15.0×10^3
		22.0×10^3	57.0×10^3	21.0×10^3
		30.0×10^3	20.0×10^3	24.0×10^3
	7	16.0×10^2	45.0×10^2	40.0×10^2
		20.0×10^2	50.0×10^2	50.0×10^2
		30.0×10^2	38.0×10^2	32.0×10^2
	14	12.0×10^2	14.0×10^2	9.0×10^2
		9.0×10^2	19.0×10^2	10.0×10^2
		10.0×10^2	7.0×10^2	10.0×10^2
	21	12.0×10^1	60.0×10^1	30.0×10^1
		50.0×10^1	20.0×10^1	90.0×10^1
		40.0×10^1	30.0×10^1	20.0×10^1
	28	80.0×10^1	50.0×10^1	1.0×10^2
		10.0×10^1	50.0×10^1	1.0×10^2
		70.0×10^1	60.0×10^1	1.0×10^2
	35	80.0×10^1	10.0×10^1	10.0×10^1
		70.0×10^1	10.0×10^1	90.0×10^1
		11.0×10^1	80.0×10^1	10.0×10^1
	42	1.0×10^2	2.0×10^2	3.0×10^2
		15.0×10^2	2.0×10^2	16.0×10^2
		5.0×10^2	21.0×10^2	6.0×10^2
30%:85% CO_2:N_2 / 11	0	34.0×10^3	77.0×10^3	15.0×10^3
		22.0×10^3	57.0×10^3	21.0×10^3
		30.0×10^3	20.0×10^3	24.0×10^3
	7	11.0×10^2	22.0×10^2	15.0×10^2
		14.0×10^2	32.0×10^2	20.0×10^2
		28.0×10^2	39.0×10^2	8.0×10^2
	14	50.0×10^4	9.2×10^4	10.9×10^4
		7.0×10^4	65.0×10^4	7.5×10^4
		42.0×10^4	39.0×10^4	64.0×10^4
	21	1.1×10^8	3.7×10^8	4.5×10^8
		3.0×10^8	3.5×10^8	4.7×10^8
		2.6×10^8	7.0×10^8	2.6×10^8
	28	80.0×10^7	1.0×10^8	90.0×10^7
		4.0×10^8	1.0×10^8	90.0×10^7
		80.0×10^7	80.0×10^7	6.0×10^8
Air / 5	0	34.0×10^3	77.0×10^3	15.0×10^3
		22.0×10^3	57.0×10^3	21.0×10^3
		30.0×10^3	26.0×10^3	24.0×10^3
	7	16.0×10^2	26.0×10^2	25.0×10^2
		22.0×10^2	20.0×10^2	18.0×10^2
		32.0×10^2	33.0×10^2	24.0×10^2
	14	7.0×10^2	10.0×10^2	16.0×10^2
		15.0×10^2	12.0×10^2	12.0×10^2

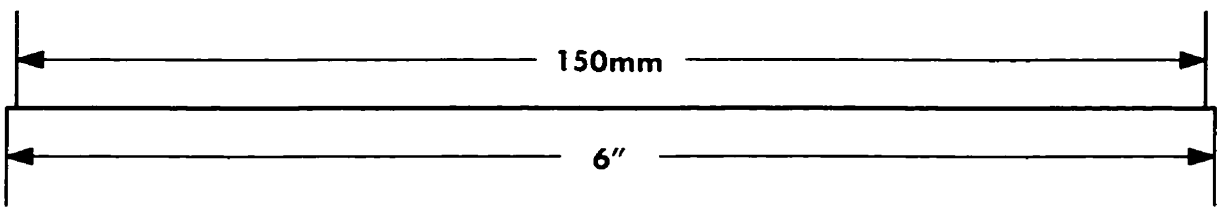
Atmosphere/ Temperature (^{0}C)	Storage time (d)	Trial no. (CFU/g)		
		1	2	3
		15.0×10^2	8.0×10^2	28.0×10^2
	21	33.0×10^1	20.0×10^1	30.0×10^1
		30.0×10^1	30.0×10^1	40.0×10^1
		20.0×10^1	50.0×10^1	60.0×10^1
	28	30.0×10^1	61.0×10^1	30.0×10^1
		40.0×10^1	30.0×10^1	40.0×10^1
		50.0×10^1	40.0×10^1	50.0×10^1
	35	20.0×10^1	10.0×10^1	10.0×10^1
		40.0×10^1	10.0×10^1	60.0×10^1
		60.0×10^1	30.0×10^1	20.0×10^1
	42	22.0×10^5	9.0×10^5	7.0×10^5
		8.0×10^5	4.0×10^5	7.0×10^5
		9.0×10^5	8.0×10^5	6.0×10^5
Air / 11	0	34.0×10^3	77.0×10^3	15.0×10^3
		22.0×10^3	57.0×10^3	21.0×10^3
		30.0×10^3	26.0×10^3	24.0×10^3
	7	10.0×10^2	10.0×10^2	50.0×10^1
		10.0×10^2	60.0×10^1	50.0×10^1
		2.0×10^2	50.0×10^1	4.0×10^2
	14	52.0×10^5	22.0×10^5	27.0×10^5
		88.0×10^5	72.0×10^5	54.0×10^5
		92.0×10^5	60.0×10^5	89.0×10^5
	21	1.3×10^7	1.7×10^7	1.4×10^7
		1.4×10^7	9.0×10^7	1.6×10^7
		1.5×10^7	1.3×10^7	1.5×10^7
	28	21.0×10^8	8.0×10^8	20.0×10^8
		30.0×10^8	12.0×10^8	20.0×10^8
		34.0×10^8	14.0×10^8	19.5×10^8

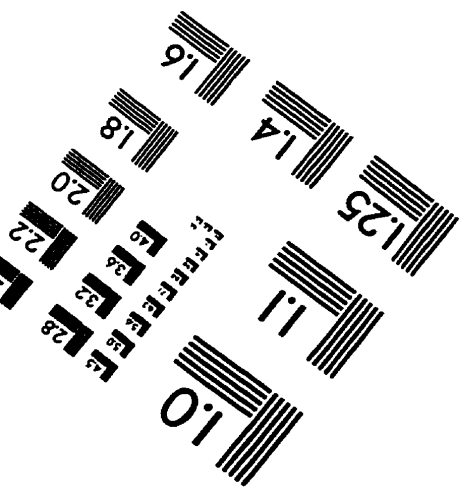
IMAGE EVALUATION TEST TARGET (QA-3)

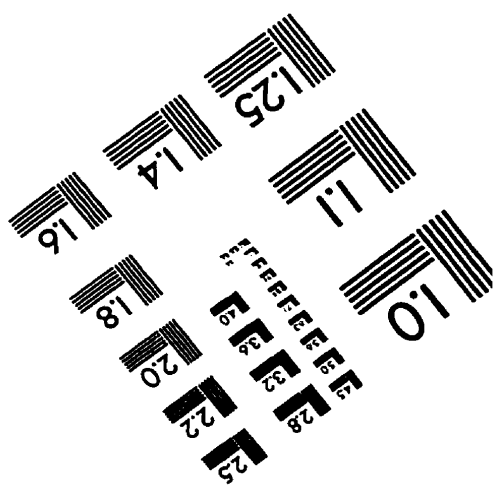












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989