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Microbial safety and quality of refrigerated trout using modified atmosphere packaging

Melanie D. Jackson

To the Graduate Council:

I am submitting herewith a thesis written by Melanie D. Jackson entitled "Microbial safety and quality of refrigerated trout using modified atmosphere packaging." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

F.A. Draughon, Major Professor

We have read this thesis and recommend its acceptance:

J. Mount, J.L. Wilson

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Dr. F.A. Draughon, Major Professor

We have read this thesis
and recommend its acceptance:

John R Mount

J. Larry Wilson

Accepted for the Council:

Carolyn Minkel

Associate Vice Chancellor
and Dean of the Graduate School

MICROBIAL SAFETY AND QUALITY OF REFRIGERATED TROUT USING MODIFIED ATMOSPHERE PACKAGING

A Thesis

Presented for the

Master of Science

Degree

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Melanie D. Jackson

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ABSTRACT

Fresh rainbow trout (*Oncorhynchus mykiss*) fillets were evaluated for microbial safety and an overall quality during storage at 3°C and 10°C. Trout fillets were packaged in a high barrier film in the following atmospheres: air, vacuum, 100% carbon dioxide, 60/40 carbon dioxide/nitrogen, and 10/85/5 carbon dioxide/nitrogen/oxygen. Trout packaged in a modified atmosphere that did not contain oxygen had lower microbial numbers at 3°C and 10°C. Fillets were considered spoiled when the aerobic plate count reached log 8 CFU/g (spoilage reference point). At 10°C, the quality of the rainbow trout began to deteriorate and show signs of spoilage (off odors) after 2 days for fillets packaged in air, vacuum, and 10/85/5 (carbon dioxide/nitrogen/oxygen). At 3°C, the packaged fillets showed signs of spoilage after 7 days for trout packaged in air and 10/85/5 (carbon dioxide/nitrogen/oxygen) and after 10 days for vacuum packaged trout. Fillets packaged in 60/40 (carbon dioxide/nitrogen) and 100% CO_2 showed signs of spoilage after day 14 of storage at 3°C. No evidence of *Salmonella* spp. or *Listeria* spp. was detected during storage of trout at 3°C or at 10°C on the fillets in the different atmospheres. The results obtained in this study demonstrated that a high concentration of CO_2 can extend the shelf life of rainbow trout for at least one week at 3°C by inhibiting the growth of spoilage microorganisms.

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CHAPTER I

INTRODUCTION

The nutritional value of fish and national emphasis on lowering fat in the diet of Americans has increased consumer demands for fish, thus increasing steadily the consumption of fish and fish products in recent years. Many of the problems associated with fish come from handling, processing, and distribution of the fish before it appears in the retail market (Reddy et al., 1992). A major problem which arises with fish is the time it takes the fish to get to the market and turnover once in the market. The process is not an automated process and distribution may take up to 2-3 days before the fish reaches the retail showcase to be sold to the consumer. A large percentage of fish (30%) was found to be spoiled when displayed in retail markets (Consumer Report, 1992).

The problems which affect the deterioration of fish are both microbiological and chemical. The deterioration process initially begins upon death of the fish because the flora on fish consists of several groups of microorganisms (Banks et al., 1980; Connell, 1980; Reddy et al., 1992). These microorganisms are naturally present on the outer surface and in the gut of the fish. While the fish is alive they are kept from invading the sterile flesh by the fish's normal defenses.

The microorganisms and/or enzymes upon death are free to interact and diffuse into the flesh where they metabolize nutrients and contribute to spoilage (Connell, 1980). Microorganisms in the flesh initially grow at a slow rate and then increase rapidly. As a consequence of this microbial action, fish take on many odors and flavors over time. Initially, they may have a sour, grassy odor followed by a rancid, putrid odor when spoilage occurs (Anon., 1985; Barnett et al., 1987). During the later stages of spoilage, microorganisms secrete proteolytic enzymes which attack the protein compounds, causing a gradual softening of the flesh (Coyne, 1932). Rancidity is another problem in fish and is caused by the attack of oxygen on unsaturated fatty acids. Fish high in fat, such as salmon and trout, are particularly susceptible to off flavors due to oxidation.

The storage temperature of fish affects the rate of multiplication of bacteria and deterioration of fish. Higher temperatures quickly give rise to undesirable flavors and odors associated with bacterial spoilage (Brown et al., 1980; Connell, 1980; Parkin et al., 1982). The lower the storage temperature, the longer the shelf-life of the fish (Reddy et al., 1992).

Although the microbiology of salt water fish and mollusks has been studied, few studies have focused on aquaculture products such as trout. Safety problems associated with the

shelf-life of trout under modified atmosphere packaging (MAP) have not been investigated.

The objective of this research was to compare the effects of air, vacuum, and modified atmospheres on shelf-life of fish and recovery of proteolytic, lipolytic, anaerobic sporeformers and pathogenic bacteria in processed trout fillets.

CHAPTER II

REVIEW OF THE LITERATURE

A. MICROFLORA OF TROUT

The microflora of trout and other fish differs depending on species, geographical location, methods of catch, season, and other environmental conditions in fish (Cox et al., 1977). The initial types of bacteria found on trout were *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, coryneform bacteria, *Micrococcus*, *Moraxella*, *Bacillus*, and *Sarcina*. The outgrowth of lactic acid bacteria on fish stored in a CO_2 atmosphere may be the result of anaerobic conditions and the more acidic medium created by the CO_2 (Banks et al., 1980).

Spoilage of fresh fish (seatrout and ocean perch) due to aerobic psychrotrophic bacteria such as the oxidase-positive Pseudomonads, is the primary problem in air permeable packages (Wolf, 1980). Banks et al. (1980) observed a higher percentage of gram-positive bacteria, especially *Lactobacillus,* in CO_2 packaged fish (Richter et al., 1983). Pseudomonads were present in high numbers in fresh fish throughout the shelf-life studies using carbon dioxide, and in some instances were equal to or exceeded all other types of bacteria present (Richter et al., 1983).

B. MICROFLORA OF FRESHWATER FISH

The microflora of freshwater fish is composed of psychrotrophic and mesophilic types of marine bacteria, such as *Flavobacterium* sp, *Achromobacter* sp, and *Pseudomonas* sp. The flesh of fresh fish is sterile, but the skin, gills and the intestines contained large concentrations of microorganisms. The types of microorganisms which are found on freshwater fish are determined by the geographical location of the fish and time of year in which the fish are caught. The microflora of fish and fish products is a function of the environment from where the fish were gathered. Fish located in the northern area had larger numbers of gram-negative psychrophilic microorganisms (*Alteromonas*, *Moraxella*, *Acinetobacter*, *Flavobacterium/Cytophaga* and *Vibrio*); whereas, in fish from warmer water there were more mesophilic gram-positive genera such as the *Micrococcus*, coryneforms, and *Bacillus* (Shewan, 1977).

Mesophilic and psychrotrophic populations on whole perch were compared at 1.1°C on MAP and ice-packed products. The psychrotrophic numbers in ice-packed fish reached $10^7/cm^2$ in 10 days; however, counts did not reach $10^7/cm^2$ until the 20th day when stored in a MAP system. The psychrotrophic count could be used as an indication of microbial stability in a MAP environment (Barnett et al., 1987).

C. STORAGE OF FISH

Factors which influenced the extension of storage shelf-life include the species of fish product, its initial quality and the nature of the finished product. They also include packaging features (headspace, permeability of the film, form or shape, and integrity) and storage temperature (Cann, 1988; Hotchkiss, 1988).

When barrier films were used in modified atmosphere (MA) preservation studies, Lannelongue et al.,(1982) noticed a reduction in CO_2 concentration of the headspace gases followed by a steady increase in the CO_2 tension. This steady increase was thought to be caused by microbial respiration (Barnett et al., 1987).

Freshwater trout can be stored for a maximum of two weeks in MAP followed by one week in refrigerated storage without seriously affecting its quality characteristics (Barnett et al., 1987; Yambrach, 1987). The refrigerated shelf-life of fresh trout can be effectively doubled, i.e., extended from 10 to 20 d, when held in "semi-permeable" type packaging in the presence of a CO_2-enriched modified atmosphere at 2°C. Microbial growth was controlled, while chemical and organoleptic changes were minimized by the improved packaging method (Barnett et al., 1987; Wilhelm, 1982). Limiting storage of trout to two weeks in the MA permitted them to be

held for an additional week outside the MA in a refrigerated environment at 2°C with minimal loss of quality. Storing the trout in the MA beyond 2 weeks significantly reduced the post-MAP shelf-life.

Dipping fish fillets in 5% potassium sorbate/10% tripolyphosphate solution and storing them under elevated CO_2 atmosphere delayed the growth and production of toxin by *C. botulinum* longer than did the use of elevated CO_2 atmosphere only (Seward, 1982). The pretreatment of MA-packaged fresh fish products with nisin provided some protection against *C. botulinum* type E growth and toxin production (Anon., 1985).

Potassium sorbate, a solution which can be used to help extend the shelf-life of fresh fish, reduced spoilage and pathogenic organisms and also inhibited the growth of trimethylamine producing bacteria in fresh fish stored at 0-1°C (Reddy et al., 1992; Wang et al., 1986). The refrigerated shelf-life of fresh trout treated with 2.3% potassium sorbate dip was effectively doubled (from 10 to 20 days) when held in "semipermeable" (laminated high/low density polyethylene bags) packages which contained CO_2 (Reddy et al., 1992).

Ammonia levels in trout stored at 1°C were not affected by storing in 80% CO_2 for 25 days. The relatively low storage temperature probably discouraged growth of ammonia-forming

organisms associated with the spoilage of fish, although spoilage odors other than ammonia were noted in the control samples near the end of the 14th day of storage (Barnett et al., 1987). Liston et al., (1963) reported that the large quantity of ammonia produced during fish spoilage was due to oxidative deamination of non-protein nitrogenous compounds of the fish tissue (Richter et al., 1983).

In some vacuum or MA-packaged fish, *Clostridium botulinum* toxin may develop before the fish would be rejected with absolute certainly (Banks et al., 1980). The hazard was even more dangerous if the rejection was on the basis of odor rather than appearance (Baker et al., 1990; Banks et al., 1980). The packaged fish could accidently be opened and placed upon human or food contact surfaces so that the toxin could be transferred to the food handler before the cooking process. Even if the fish was considered inedible and discarded after it had been smelled, the toxin could already have been transferred to an unsuspecting victim through handling the contaminated fish (Post et al., 1985).

D. MODIFIED ATMOSPHERE PACKAGING OF FISH

Modified Atmosphere Packaging (MAP), a technologically viable method for packaging food has been developed as a supplement to ice and mechanical refrigeration to reduce the losses and extend the storage shelf-life of fresh seafood

products (Richter et al., 1983). A typical modified atmosphere packaging system involves the removal of air from an oxygen-impermeable container, injection of a desired amount of gas mixture (usually CO_2), followed by sealing of the container. The use of CO_2 as a preservative for fresh proteinaceous food is not a recent technique. The MAP gas mixtures replace the normal air ratios to help regulate microbial activity and/or retard deterioration of the product. MA packaging dates back to the 1930's, when it was discovered that the shelf-life of fish could be extended in an atmosphere containing 100% CO_2. This method of packaging prolongs the shelf-life of fish and uses a carbon dioxide rich atmosphere which selectively inhibits growth of bacteria (Coyne, 1932). Modified atmosphere packaging (MAP) uses high barrier films with low rates of transmission for oxygen (O_2), carbon dioxide (CO_2), and nitrogen (N_2) for packaging of fresh seafood products (Reddy et al., 1992).

CO_2, inhibits aerobic bacterial, yeast and mold activity in foods by increasing the lag phase and generation time of the bacteria. It has been used by the food industry for many years, particularly for preservation of highly perishable, high-value commodities (Brecht, 1980). Carbon dioxide can decrease the growth rate and delay growth of the spoilage organisms (Finne, 1982; Genigeorgis, 1985). Clark and West

(cited by Silliker 1982) stated that if CO_2 were present continuously, microbial growth could be reduced.

At high levels, CO_2 effectively decreased overall microbial growth in different products (Genigeorgis, 1985). In addition, high levels of CO_2 in modified atmospheric packaging may cause undesirable organoleptic changes in some packaged fresh fishery products (Statham, 1984). Organoleptic changes including odor, taste, texture, and flavor, can occur in fishery products stored at high CO_2 atmospheres (Gray et al., 1983). The microflora in foods packaged with CO_2 increased as the storage temperature increased.

When CO_2 dissolves into the fish tissue, it causes deformation or collapse of the package, and may affect the color of the product by interacting with pigment in the flesh (Daniels et al., 1985). Concentrations of CO_2 in the flesh of the MAP trout samples increased from 67 ppm in controls to 988 ppm the sixth day of storage in fresh trout packaged under 80% CO_2 and 20% N_2. The solubility in muscle was dependent on CO_2 tension in the MA. As CO_2 is absorbed into the flesh of the fish, other chemical/physical changes occur with muscle protein, e.g., complexing of amino (NH_2) groups with CO_2 (Silliker, 1982). The decrease of CO_2 after the 6th day of

storage was attributed to reduce pCO_2 in the semi-permeable bag and the ability of fish tissue to buffer (Barnett et al., 1987).

An increase in CO_2 would inhibit metabolic activity of the microflora and favor the growth of microaerophilic bacteria that favor lower oxygen tension (Cavett, 1967). Fish packaged without CO_2 displayed a typical population of gram-negative organisms during storage while fish packaged in CO_2 exhibited a higher population of gram-positive organisms, specifically *Lactobacillus* (Banks et al., 1980).

Lannelongue et al., (1982) found 100% CO_2 to be an effective method for limiting bacterial growth using modified atmosphere packaging in finfish fillets. There was an overall decrease in both growth rate and total bacterial count observed in 100% CO_2 treatments comparison to fish with no gas treatment. The results of various studies using CO_2 to inhibit spoilage bacteria are shown in Table 1.

E. OTHER GASES

To prevent deformation and collapsing of the food package as CO_2 is absorbed, it can be used in combination with other gases such as N_2 and O_2 (Cann et al., 1983).

N_2, a chemically inert gas and filler, can be used to balance mixture or to reduce the concentration of the

Table 1. Shelf life obtained by using modified atmosphere packaging.

Author	Atmosphere	Species	Storage temperature (°C)	Shelf-life (days)	Shelf-life extension (%)
Coyne (1933)	100% CO_2	Cod, whiting, haddock	0	10-12	50-100
Stansby & Griffiths (1935)	25%CO_2	Haddock	0	25	100
Fey (1980)	60%CO_2:21%O_2: 19%N_2	Red hake	0-1	>27	-
	60%CO_2:5%O_2: 35%N_2	Red hake	0-1	>27	-
	20%CO_2:21%O_2: 59%N_2	Red hake	0-1	>27	-
Lee (1982)	20%CO_2:20%O_2: 60%N_2	Dover sole	0		100
	60%CO_2:20%O_2: 20%N_2	Dover sole	0		200
Stier et al., (1981)	100%CO_2	King salmon	4.4	12	100
	100%CO_2	King salmon	22	<2	<100
Barnett et al., (1982)	90%CO_2:10% air	Chum and Coho salmon	0	>21	-

Author	Atmosphere	Species	Storage temperature (°C)	Shelf-life (days)	Shelf-life extension (%)
Coyne (1933)	100% CO_2	Cod, whiting, haddock	0	10-12	50-100
Stansby & Griffiths (1935)	25%CO_2	Haddock	0	25	100
Haard and Lee (1982)	100%CO_2	Salmon	3	>20	>100
Mills & Tiffney (1982)	40%CO_2:30%N_2: 30%O_2	Various white fish	0 2 5	15 8 6	70 60 50

other gases in a mixture (Genigeorgis, 1985; Statham, 1984). It has a minimal effect on the bacterial growth and shelf-life of fishery products and has no reactions with other food components (Statham, 1984). N_2 also keeps the package from collapsing as CO_2 dissolves into the product (Reddy et al., 1992).

O_2 can be used along with CO_2 and N_2 in modified atmosphere packaging in the same or different ratios as it naturally occurs in the atmosphere. It also maintains the oxygenated form of myoglobin, which prevents the irreversible conversion of myoglobin to metmyoglobin in red meats which improves color and shelf-life (Genigeorgis, 1985). However, use of O_2 is not necessary for maintaining color of fish. Oxygen may retard growth of some anaerobic spoilage bacteria (Statham, 1984); however, this has not been proven conclusively. When the oxygen levels were decreased by the application of a vacuum, strict aerobic types of bacteria did not grow (Banks et al., 1980; Barnett, et al., 1982). Therefore, facultative anaerobes, microaerophiles, and strict anaerobes increased as the levels of CO_2 increased. This accounts for the different types of microflora found on fish packaged in CO_2 and without CO_2 (Banks et al., 1980).

F. SAFETY CONSIDERATION

Spoilage is the single most important factor in preventing foodborne illness because it is often spoilage that warns the consumer that a given food may be inedible (Hotchkiss, 1989). The major safety concern in MAP fish has been growth and toxin formation by the non-proteolytic clostridia which can grow at temperatures as low as 3.3°C (Kautter, 1964). *C. botulinum* is frequently found in marine and freshwater environments (Skura, 1988). There has been controversy as to whether or not *C. botulinum* toxin production can or cannot occur before organoleptic spoilage at room temperature or above (Hotchkiss, 1988). Under certain conditions such as temperature abuse, or even at 8°C under an anaerobic MA, there was no doubt that botulinum toxin could be detected well before evidence of organoleptic deterioration (Post et al., 1985). Because the proper refrigeration of foods throughout the distribution chain cannot be ensured, some experts feel that consumer sized packages of MAP fish should not be available for sale at the retail level (Farber, 1991).

Modified atmosphere maintains quality by suppressing normal spoilage bacteria in products stored at refrigeration temperatures. Safety concerns may arise from storage of MA packaged products for long periods of time because of the potential for increasing growth of certain foodborne

pathogens. Safety was a particular concern when storage temperatures were not strictly monitored (Genigeorgis, 1985).

Reddy et al., (1992) suggested that barrier systems should be constructed on a product-to-product basis and evaluated by using inoculated pack studies which establishes safety limits in all MA packaged products. The products would be packaged under conditions similar to those of commercial MA packaging, subjected to refrigerated temperatures and long-term temperature abuse, and designed to evaluate shelf-life, organoleptic qualities, growth, and toxin production by *Clostridium* and psychrotrophic pathogens (Reddy et al., 1992).

Strict temperature control is critical in minimizing safety risks of vacuum and MA packaging. Various attempts have been made to ensure continued refrigerated conditions (Reddy et al., 1992). Under low O_2 atmospheres and refrigeration temperatures, spores of non-proteolytic *C. botulinum* can grow and produce toxins in MA packaged products without causing organoleptic signs of spoilage (Reddy et al., 1992; Stier et al., 1981). Baker and Genigeorgis (1990) also reported that temperature was the most important variable for determining safety of MA packaged fish, since it has the greatest effect on *C. botulinum* growth and toxigenesis. *C. botulinum* type E grew and produced toxin at temperatures as low as 3.3°C (Reddy et al., 1992). Since many fish products rely heavily or solely on refrigeration for protection from foodborne

botulism, strict adherence to temperature controls is necessary not only by the food producers, distributor, and retailer, but also by the consumer, who has the ultimate and final responsibility for safety (Liciardello, 1976; Ogrydziak et al., 1982). Some of the MA-packed fresh fishery products may need to be stored below 0°C for optimal shelf life and complete protection from risk of foodborne botulism (Lilly et al., 1990). As the storage temperature decreased, the time for toxin development and organoleptic spoilage increased in fresh products regardless of the MA under which they were packaged (Reddy et al., 1992; Veranthe et al., 1979). The need for temperature control for product safety and the likelihood of temperature abuse have been two major concerns of regulatory agencies (Reddy et al., 1992; Statham, 1985).

Farber (1991) concluded the only effective way to assure the safety of refrigerated vacuum-packaged or MAP fish products would be to either: (a) keep the product below 3°C at all times, (b) heat the product sufficiently to destroy spores of all strains, or (c) heat the product sufficiently to inactivate the non-proteolytic spores and then keep the product well below 10°C. The latter two points may be effective from a theoretical standpoint, but in practice it may be difficult in a fish processing environment to avoid post-processing contamination with spores of *C. botulinum*.

CHAPTER III

MATERIALS AND METHODS

A. TROUT SAMPLES

Rainbow trout fillets were purchased at Crosseyed Cricket, which is a local trout and catfish farm located approximately 86 km from the University of Tennessee, Knoxville. The trout fillets were placed on ice initially at the fish farm and conveyed back to the University where the fillets were stored at 4°C for 14 hours until microbial analyses were conducted.

B. SAMPLE PACKAGING

Trout fillets were placed on a styrofoam tray, (4M, LinPac, Wilson, NC) and packaged in high barrier film bags, BDF-2001 (CryoVac, W.R. Grace & Co., Simpsonville, SC). The oxygen transmission rate of the barrier film was 5.0 cc/sq.M./24 hours. Fillets were initially vacuum packaged using a vacuum packaging machine, Model 8210-2 (CryoVac, W.R. Grace & Co., Woburn, MA.). A septum (Modern Control Incorporate, Minneapolis, MN) was placed on all the packaged fillets, except the fillet which were to be stored under a vacuum. All other packaged fillets were backflushed with air or an appropriate modified atmosphere (100% CO_2, 60/40 carbon

dioxide/nitrogen, 10/85/5 carbon dioxide/nitrogen/oxygen). Gas analysis was not performed on vacuum packaged samples since there was no measurable gas interface between package and product. All styrofoam trays were vacuum packaged before use to reduce the level of oxygen in the styrofoam except those trays which were packaged in an air atmosphere.

C. PHYSICAL/CHEMICAL ANALYSES

Gas Analyses

The packaged fillet (air, 100% CO_2, 60/40, carbon dioxide/nitrogen, 10/85/5, carbon dioxide/nitrogen/oxygen) was placed in a pan of tap water with the package submerged for gas sampling. A 5.0 mL needle (gauge) was placed into the septum of the packaged fillet to collect gases in the packaged sample. A gas chromatograph, Model 5890A Series2A (Hewlett Packard, Wilmington, DE) was utilized to analyze the samples. A CTRL column (Alltech Associates, Inc., Deerfield, IL), 1.82 meters in length, was used with a helium flow rate of 65 ml/min at 70°C. The chromatograph was equipped with a thermal conductivity detector. The percentages of oxygen, carbon dioxide, and nitrogen were determined from a standard curve using Scotty I analyzed calibration gases.

pH

The pH readings were taken when the trout samples were initially opened to begin the microbiological analyses. A Fisher Accumet pH meter, Model 825MP (Fisher, Scientific, Fair Lawn, NJ), equipped with a surface probe was utilized. The pH was determined to the nearest tenth.

Quality Evaluation

The overall quality evaluation of the fillets was based upon the scale of Barnett et al., (1987). The scale included organoleptic changes perceived with the senses such as appearance, odor, texture and color.

D. MICROBIOLOGICAL ANALYSES

Microbiological media

All media prepared for this research was obtained from Difco Laboratories (Detroit, MI), unless otherwise stated.

Microbiological counts

Twenty five grams of sample were added to 225 mL (1:10) of Butterfield's diluent in a Seward Medical Stomacher 400 Closure Bag (Tekmar, Cincinnati, OH). The sample was mixed

in a Stomacher Laboratory Blender 400 (Tekmar, Cincinnati, OH) for 120 seconds. Serial dilution were used to determine the following microbial counts: aerobic plate count using standard method agar, incubated at 32°C for 48 hours; psychrotrophic plate count, standard methods agar, incubated at 4°C for 10 days; coliforms, violet red bile agar, incubated at 32°C for 24 hours; lactics, lactobacillus, incubated at 32°C for 48 hours; lipolytic, spirit blue agar, incubated at 32°C for 48 hours; proteolytic, standard method caseinate agar, incubated at 32°C for 48 hours and transferred to 7°C for 10 days; yeast and mold, rose bengal agar (Oxid Division, Columbia, Maryland).

Aerobic Sporeformers

To enumerate aerobic sporeformers, 20 grams of the fillet were added to 200 mL of Butterfield's diluent (1:10). The sample was mixed in a Stomacher Laboratory Blender 400 for 120 seconds. Fifty grams of sample were transferred into 30 ml of trytone glucose extract agar. The mixture was kept in a 80°C water bath for 20 minutes. Next, it was distributed into six plates in equal amounts and the plates were incubated at 35°C for 48 hours.

Anaerobic Sporeformers

To evaluate anaerobic sporeformers, the Fung's Double Tube Method was used (Fung et al., 1980). One mL of sample was placed into a tube which contained approximately 30 ml of hot Brewer's medium. A sterile test tube was placed inside the outer tube and covered with a screw cap. The sample was incubated at 35°C for 48 hours.

***Listeria* enumeration**

Twenty five grams of trout were added to 225 mL of University of Vermont (UVM) I (Oxoid Division, Columbia, Maryland) enrichment broth and mixed in a Stomacher Laboratory Blender 400 for 120 seconds. Samples were incubated at 30°C for 48 hours. One tenth mL of the University of Vermont (UVM) I enrichment broth was transferred into 10 ml of University of Vermont (UVM) II (Oxoid Division, Columbia, Maryland) and incubated at 30°C for 48 hours. The UVM II broth was streaked on PALCAM (Oxoid Division, Columbia, Maryland) agar and incubated at 30°C for 48 hours for *Listeria* isolates.

***Salmonella* enumeration**

Twenty five grams of trout were added to 225 mL of Tertathionate enrichment broth and mixed in a Stomacher Laboratory Blender 400 for 120 seconds. Samples were

incubated at 42°C for 48 hours. The enrichment broth was streaked onto XLT4 agar with novobiocin and incubated at 35°C for 48 hours.

E. EXPERIMENTAL DESIGN

Samples were stored at 3°C and 10°C. The sampling days for 3°C were 0, 3, 7, 10, 14, and 21 days. At 10°C, the sampling days were 0, 2, 4, 6, and 8 days. Samples were analyzed until log 8 CFU/g was reached on the aerobic plate count at 3°C. At this point sampling was discontinued and trout samples were considered spoiled. Three replications were performed at each temperature. Five different atmospheres were used for MA packaging of the rainbow trout fillets: air, vacuum, 100% carbon dioxide, 60/40 carbon dioxide and nitrogen, 10/85/5 carbon dioxide, nitrogen, and oxygen.

F. STATISTICAL ANALYSES

Data were analyzed using a randomized split block design. Mean values were calculated from three replications at 3°C and 10°C. The General Linear Model of SAS (1985) was used to show the effects of storage atmospheres, storage time, storage temperature, and the interactions. The Student Newman Keuls test of SAS was used to separate and compare the mean values.

CHAPTER IV

RESULTS AND DISCUSSION

A. Microbiological counts

Aerobic plate count

The initial aerobic plate count (APC) on rainbow trout fillets was between log 2 and 4.5 CFU/g fish (Figures 1 and 2). *Salmonella* spp. and *Listeria* spp. were not detected in rainbow trout fillets. During storage of the samples at 10°C, the APC on trout increased to approximately log 8 CFU/g (spoilage reference point) in 6 days for air, vacuum and 10/85/5 (carbon dioxide/nitrogen/oxygen) packages and 8 days for fillets packaged in 100% CO_2 and 60/40 (carbon dioxide/nitrogen)(Figure 1). In samples held at 3°C, the APC reached approximately log 8 CFU/g in 14 days for air, vacuum, and 10/85/5 (carbon dioxide/nitrogen/oxygen) packages and 21 days for the 60/40 (carbon dioxide/nitrogen) and 100% CO_2 packages. The APC results indicated the importance of strict temperature control in combination with a modified atmosphere packaging. At 3°C, the 100% CO_2 and 60/40 (carbon dioxide/nitrogen) delayed spoilage of the fillets at least 7 days (Figures 2). The microbiological effect of the CO_2 modified atmosphere without the presence of oxygen on the APC was evident by the prolonged lag phase during the first 1-2

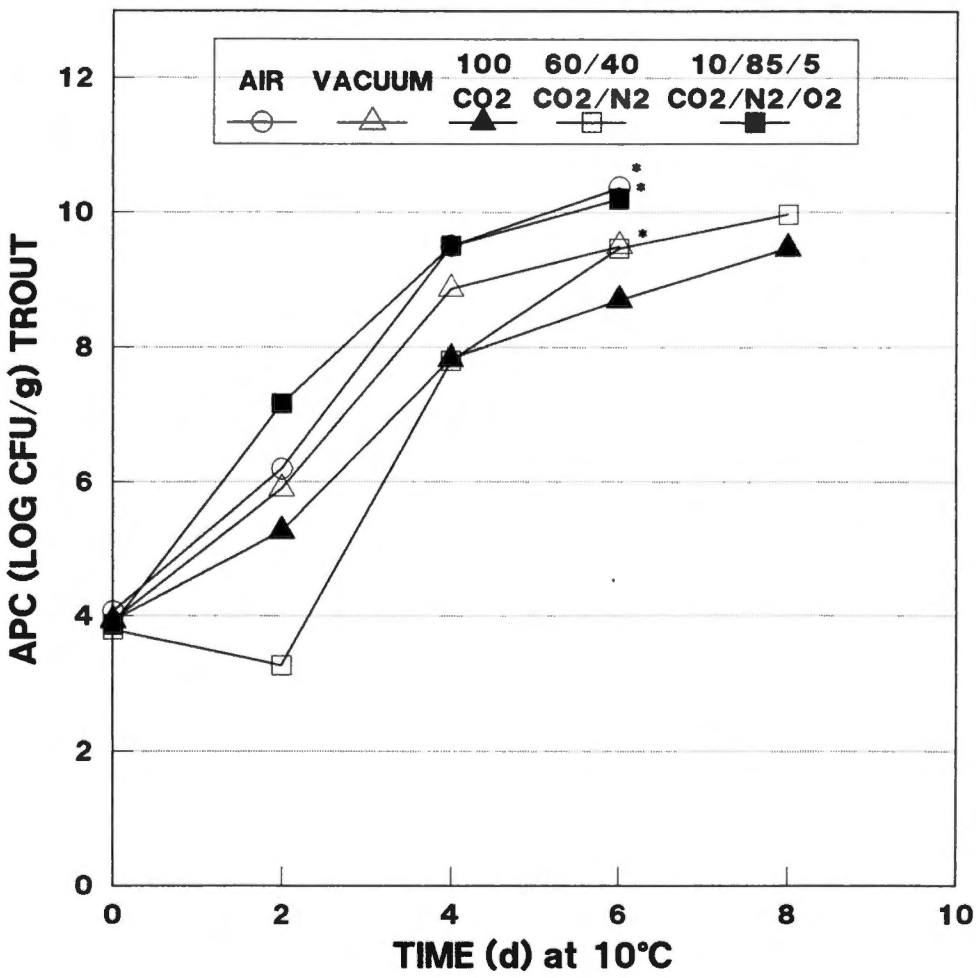

Figure 1. Aerobic Plate Count of trout fillets packaged under air, vacuum, and modified atmospheres at 10°C.
("*" point at which sampling was discontinued, samples were considered spoiled.)

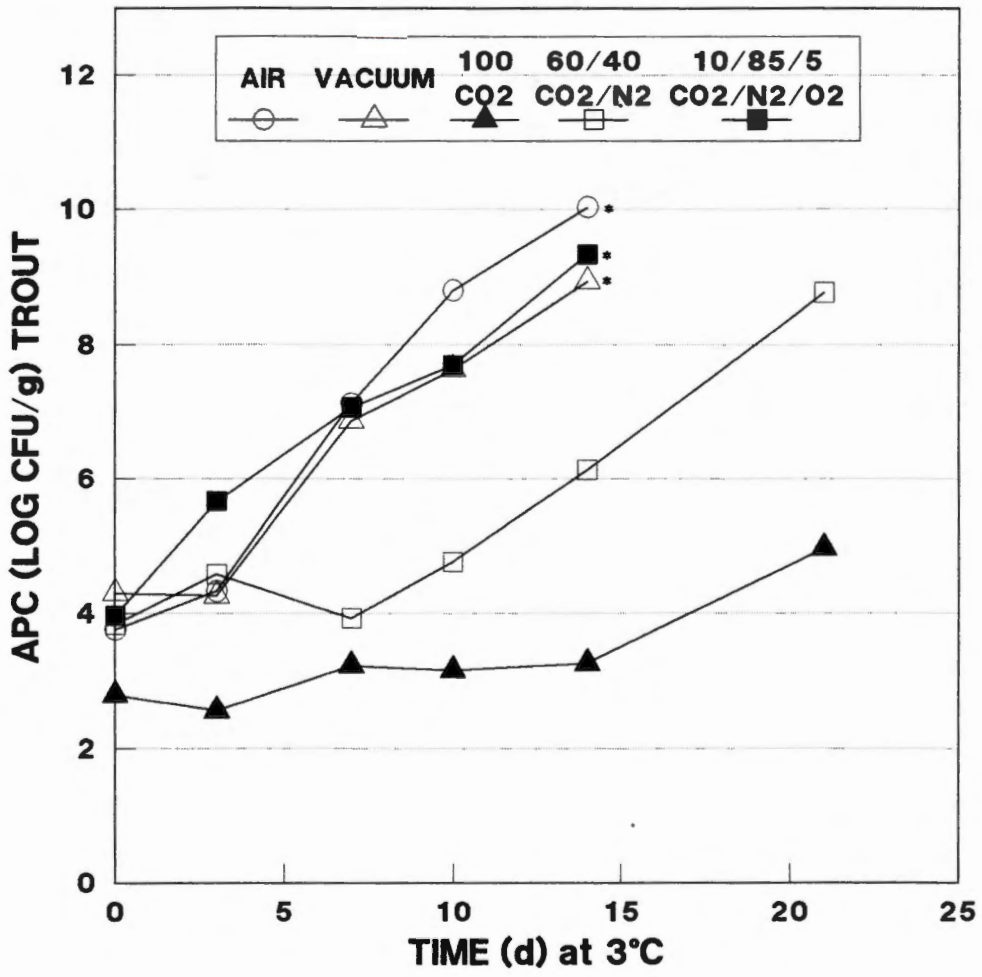

Figure 2. Aerobic Plate Count values of trout fillets packaged under air, vacuum, and modified atmospheres at 3°C.
("*" point at which sampling was discontinued, samples were considered spoiled.)

days of storage at 10°C and during the first 7-14 days of storage at 3°C (Figures 1 and 2).This microbial effect can be attributed to the increase in CO_2 since vacuum packages were also low in O_2.

The atmosphere of the package had a significant effect on the APC and was found to be significant ($p < 0.05$) at 3°C and 10°C (Appendixes A1 and A2). The mean APC's for trout stored in air, vacuum, 100% CO2, 60/40 (carbon dioxide/nitrogen), and 10/85/5 (carbon dioxide/nitrogen/oxygen) were 7.5, 7.1, 6.4, 6.1, 7.7 log CFU/g at 10°C and 6.8, 6.4, 4.5, 4.6, 6.7 log CFU/g at 3°C, respectively (Appendixes A3 and A4).

Psychrotrophic bacteria

The growth of psychrotrophic organisms in rainbow trout fillets held at 10°C closely resembled that of the aerobic organisms (Figures 1-3), suggesting the aerobic plate count consisted primarily of psychrotrophs. The pattern of growth for fillets stored at 3°C was not consist with the aerobic plate count at 3°C although the atmosphere within the trout package had a significant effect on psychrotrophic plate count (PPC) at both temperatures similar to the aerobic plate count ($p < 0.05$) (Appendixes A5 and A6). The initial PPC ranged

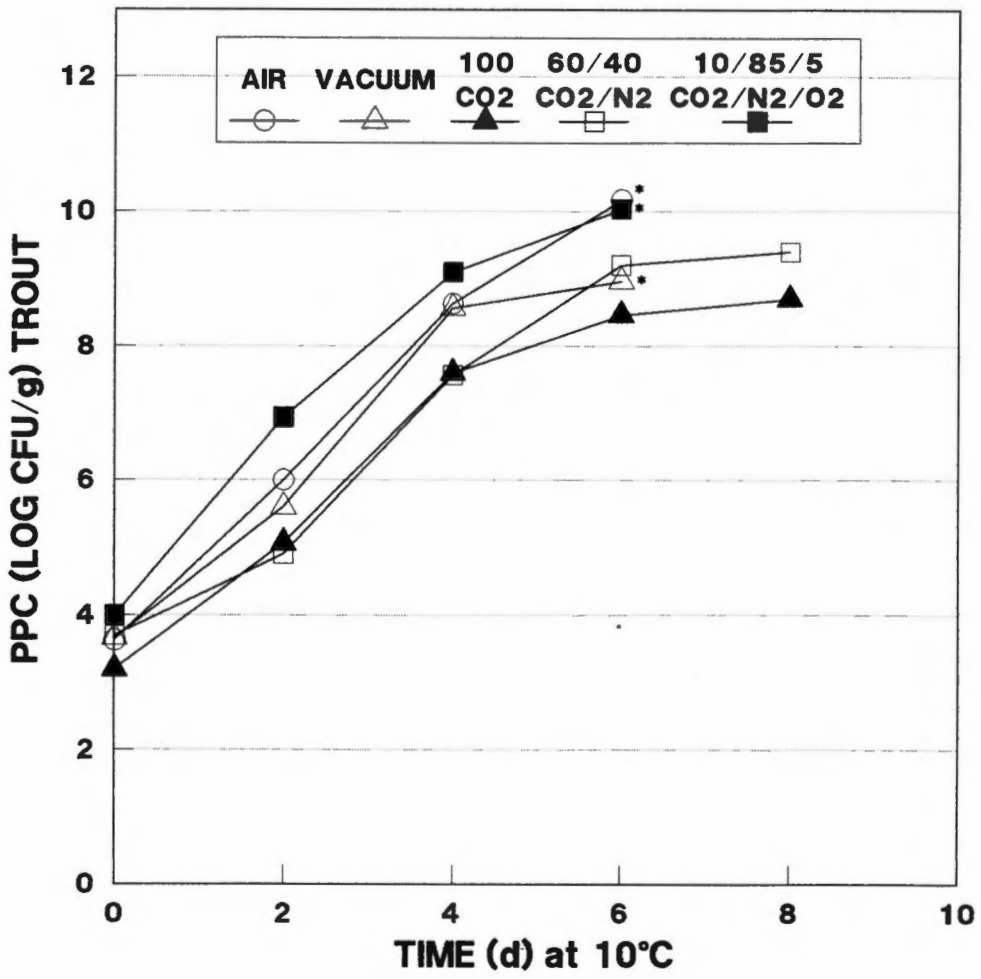

Figure 3. Psychrotrophic Plate Count of trout fillets packaged under air, vacuum, and modified atmospheres at 10°C.
("*" point at which sampling was discontinued, samples were considered spoiled.)

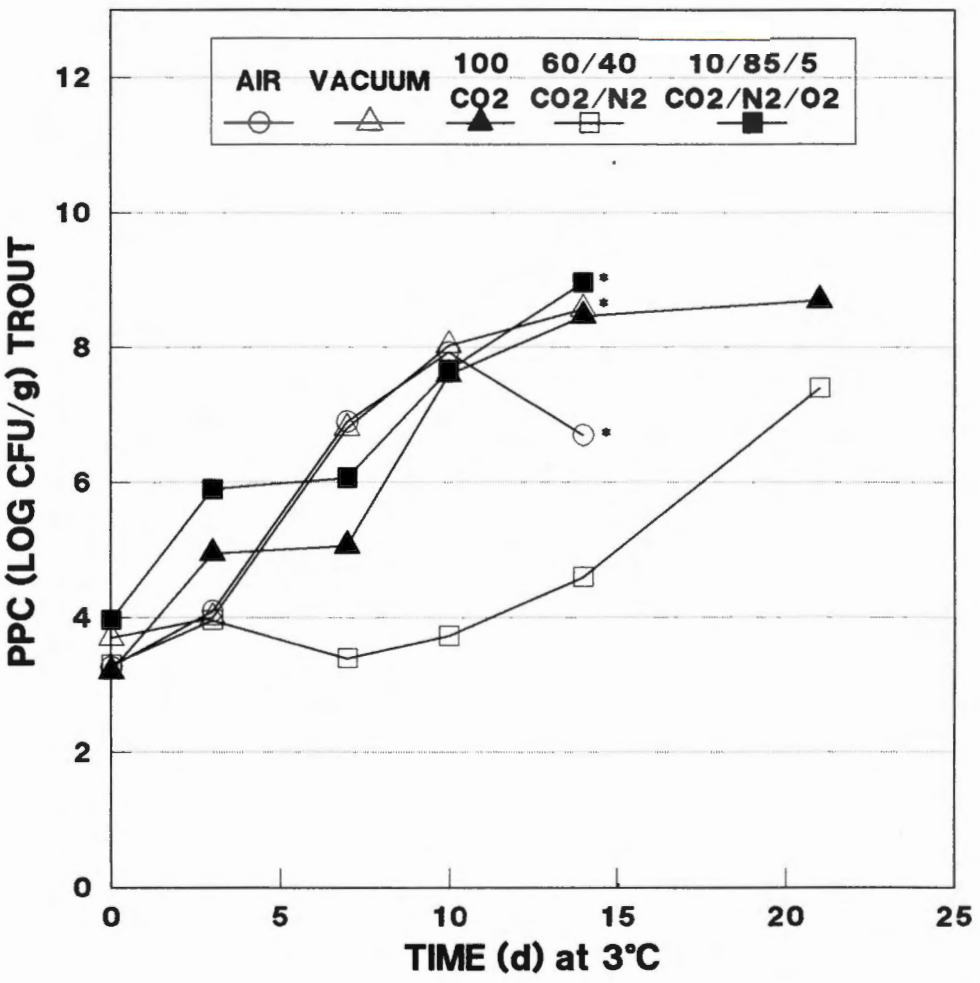

Figure 4. Psychrotrophic Plate Count of trout fillets packaged under air, vacuum, and modified atmospheres at 3°C.
("*" point at which sampling was discontinued, samples were considered spoiled.)

between log 3 and 4 CFU/g but reached levels approximately equal to APC by day 2 at 10°C and day 3 at 3°C (Figures 3 and 4).

Coliform bacteria

The initial levels of coliform bacteria ranged between log 2 and 4 CFU/g at 10°C and 3°C (Figures 5 and 6). Coliform levels reached log 9.2, 8.5, 8.0, 8.8, and 9.2 CFU/g at 10°C in fish packaged under air, vacuum, 100% CO_2, 60/40 (carbon dioxide/nitrogen), and 10/85/5(carbon dioxide/nitrogen/ oxygen); respectively (Figure 5). Coliform levels in fish stored at 3°C reached log 5.4, 4.8, 4.5, 5.9 and 7.9 CFU/g in air, vacuum, and CO_2 gas mixes, respectively (Figure 6). The 60/40 (carbon dioxide/nitrogen) atmospheres decreased coliform bacteria during the first two days of storage at 10°C, and the same effect was evident during the first 10 days of storage at 3°C for both the 60/40 and 100% CO_2 atmospheres (Figures 5 and 6).

The atmosphere of the package had a significant effect upon the growth of coliforms in trout samples at 3°C ($p < 0.05$) (Appendixes A7) with the 60/40 (carbon dioxide/nitrogen) and 100% CO_2 packages having the significantly lower mean coliform count (Figure 6).

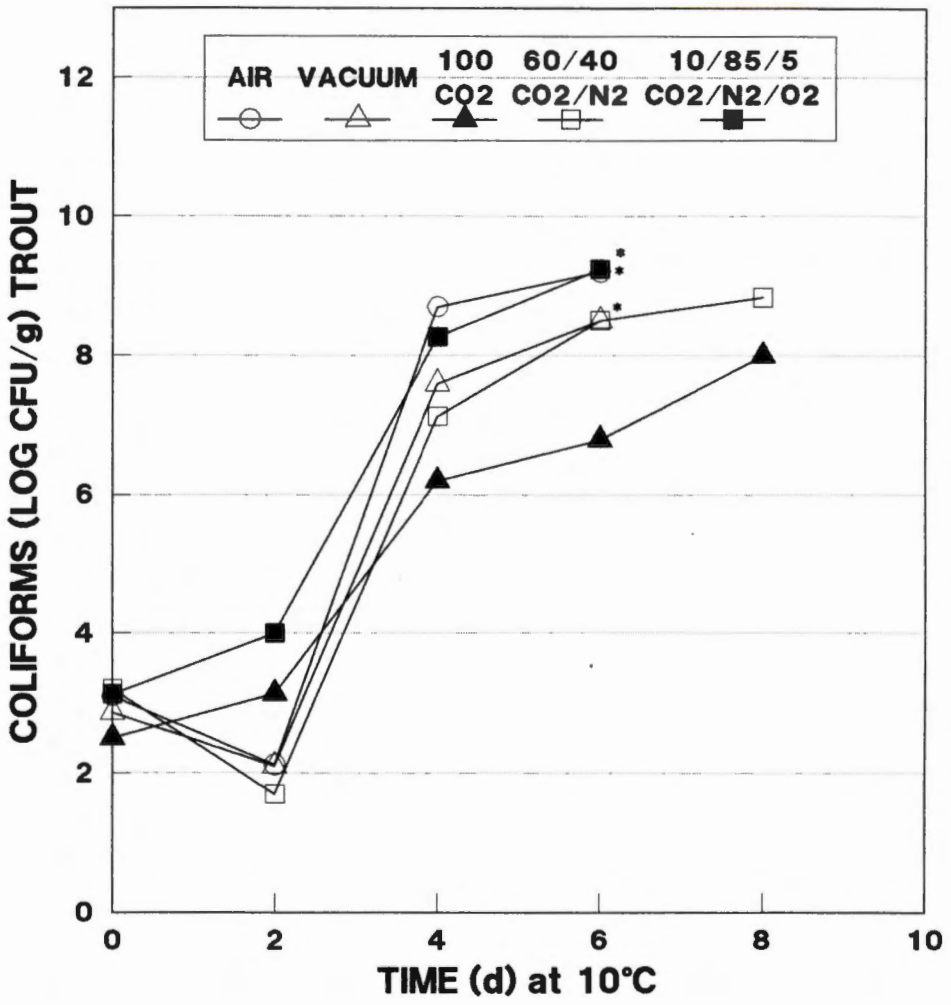

Figure 5. Coliform count of trout fillets packaged under air, vacuum, and modified atmospheres at 10°C. ("*" point at which sampling was discontinued, samples were considered spoiled.)

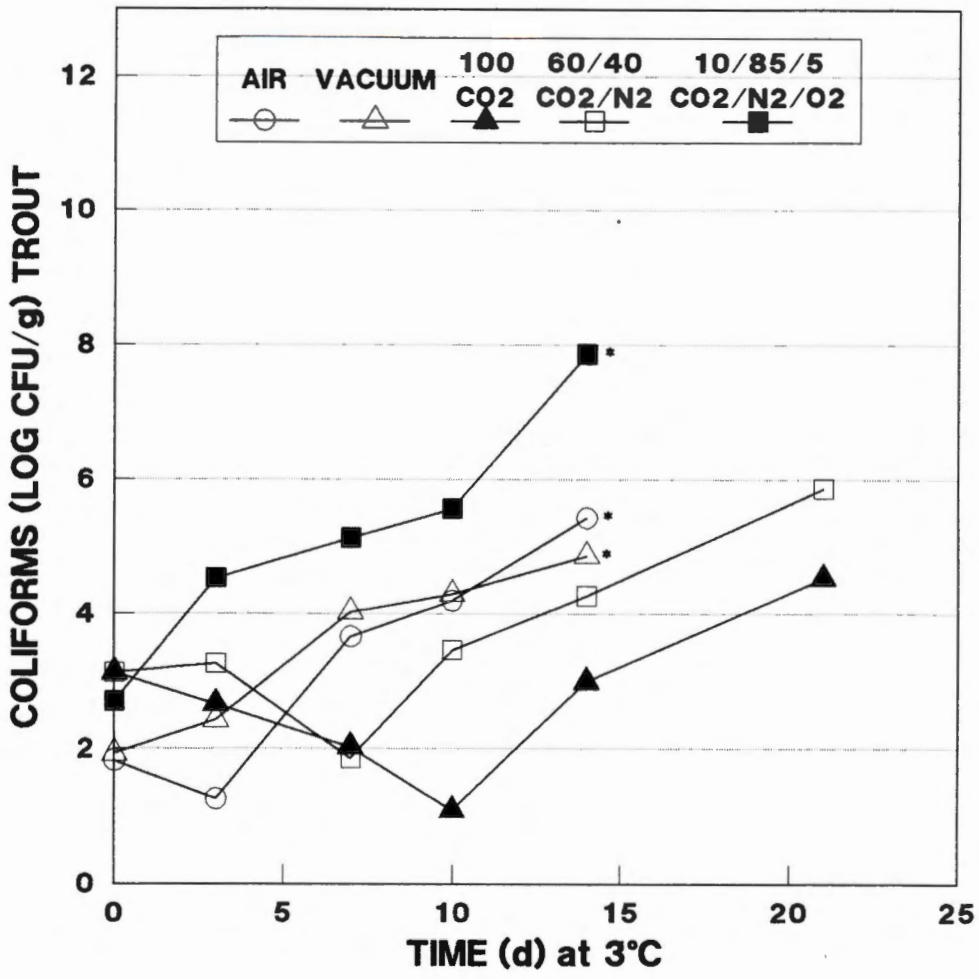

Figure 6. Coliform count of trout fillets packaged under air, vacuum, and modified atmospheres at 3°C. ("*" point at which sampling was discontinued, samples were considered spoiled.)

Lactic acid bacteria

Atmosphere had a significant effect on the growth of lactic acid bacteria at 10°C and 3°C ($p < 0.05$) (Appendixes A9 and A10). The mean lactic acid counts for fish packaged under 60/40 (carbon dioxide/nitrogen) and 100% CO_2 were significantly lower ($p < 0.05$) than the mean lactic counts for fish packaged under 10/85/5 (carbon dioxide/nitrogen/oxygen) atmospheres (Appendixes A3 and A4). The lactic acid bacterial count on the rainbow trout fillets initially ranged from log 3 to 4 CFU/g. At 10°C, lactic acid bacteria in the trout reached approximately log 8 CFU/g by day 6 in all treatments. At 3°C the number of lactic acid bacteria in trout packaged under air, vacuum, and 10/85/5 (carbon dioxide/nitrogen/ oxygen) reached approximately log 8 CFU/g by day 12. The 100% CO_2 and 60/40 (carbon dioxide/nitrogen) reached approximately log 8 CFU/g by day 2 (Figures 7 and 8). The CO_2 containing atmospheres increased the lag phase by two days at 10°C and day seven days at 3°C.

Proteolytic

The 60/40 (carbon dioxide/nitrogen) and 100% CO_2 atmospheres significantly ($p < 0.05$) reduced growth of proteolytic organisms in trout samples at 3°C while only the 100% CO_2 would grow at 10°C (Appendixes A11 and A12). The

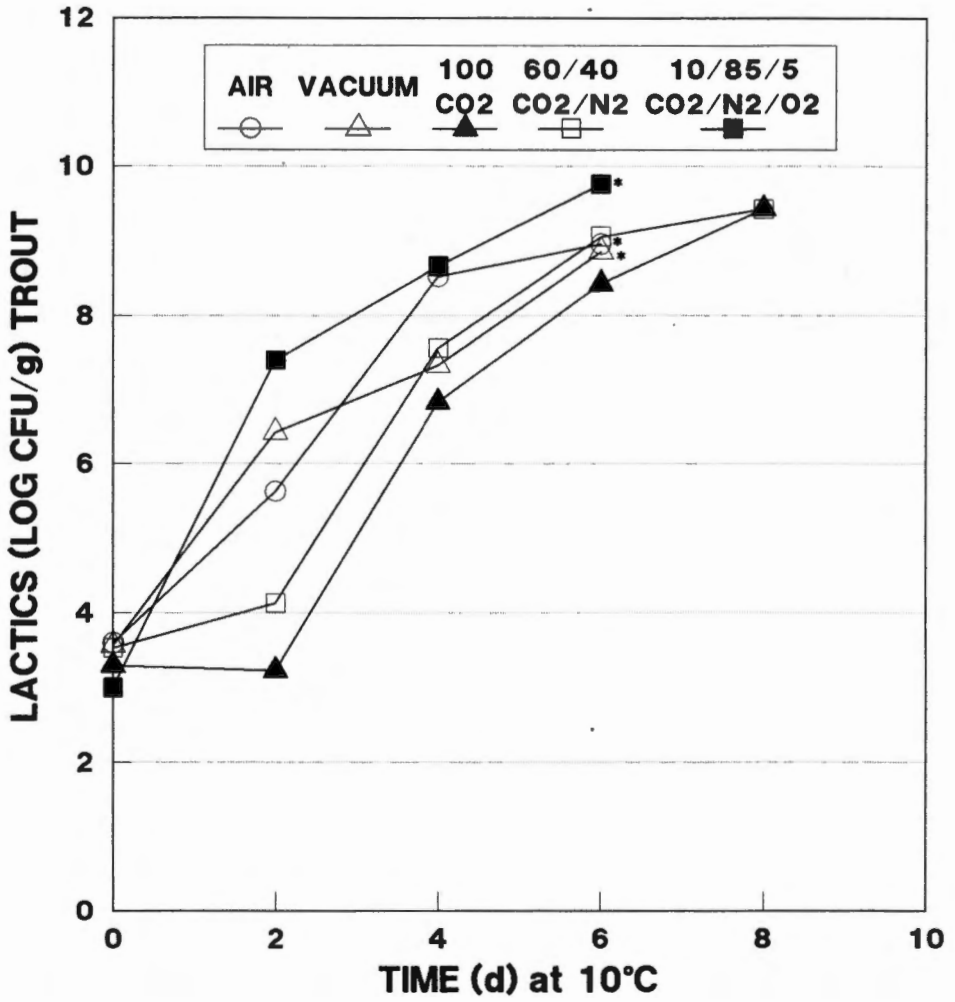

Figure 7. Lactic count of trout fillets packaged under air, vacuum, and modified atmospheres at 10°C. ("*" point at which sampling was discontinued, samples were considered spoiled.)

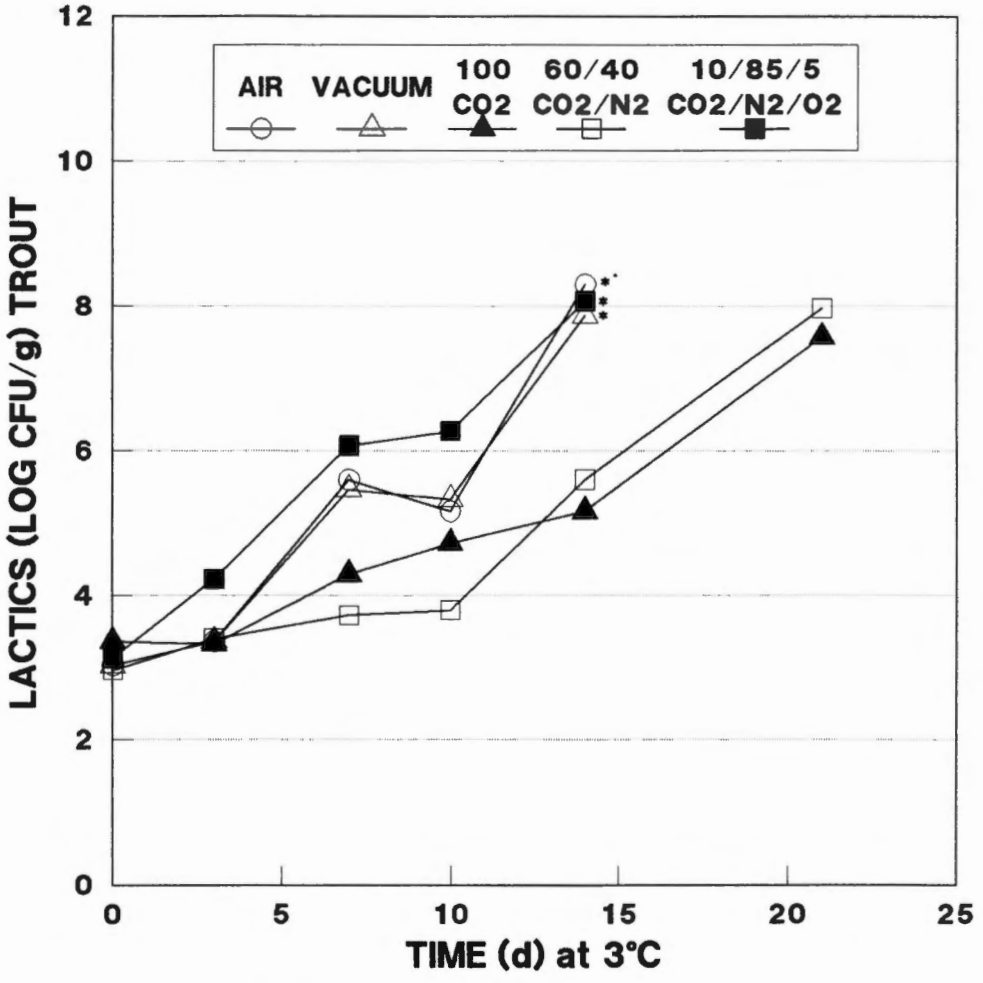

Figure 8. Lactic count of trout fillets packaged under air, vacuum, and modified atmospheres at 3°C. ("*" point at which sampling was discontinued, samples were considered spoiled.)

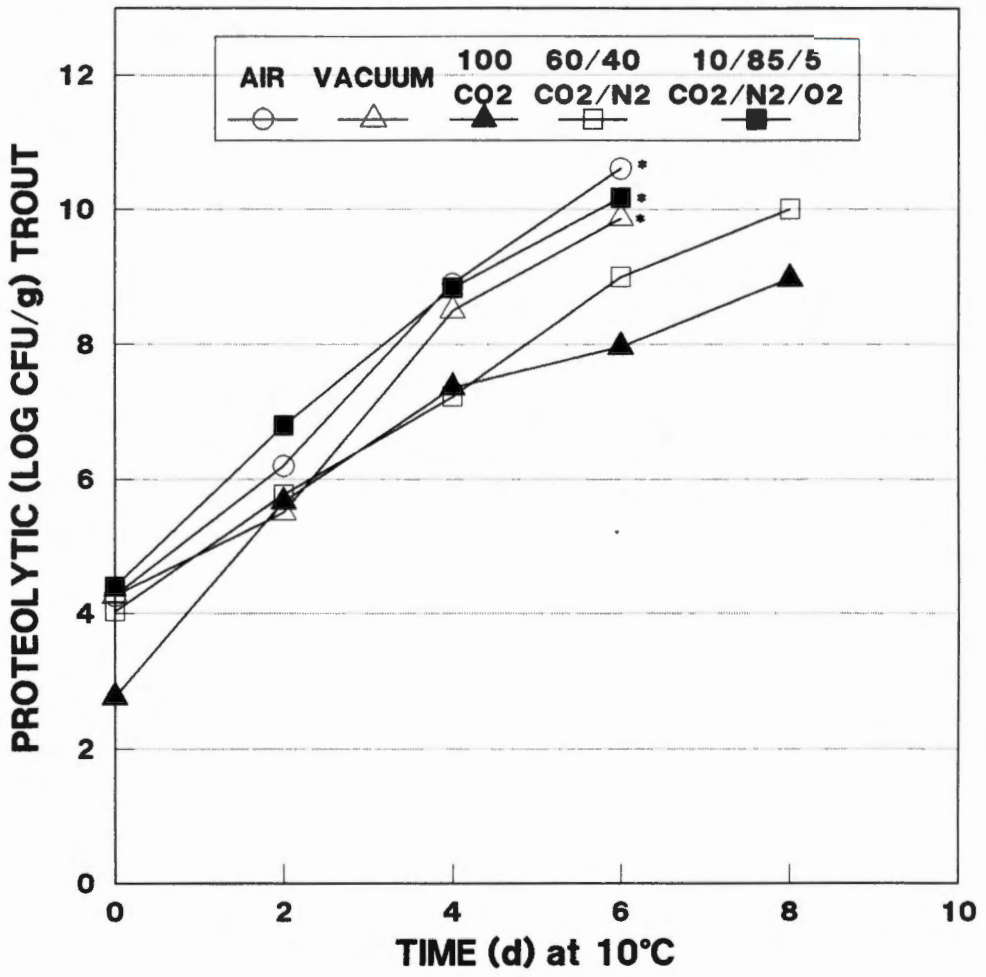

Figure 9. Proteolytic bacteria of trout fillets packaged under air, vacuum, and modified atmospheres at 10°C.
("*" point at which sampling was discontinued, samples were considered spoiled.)

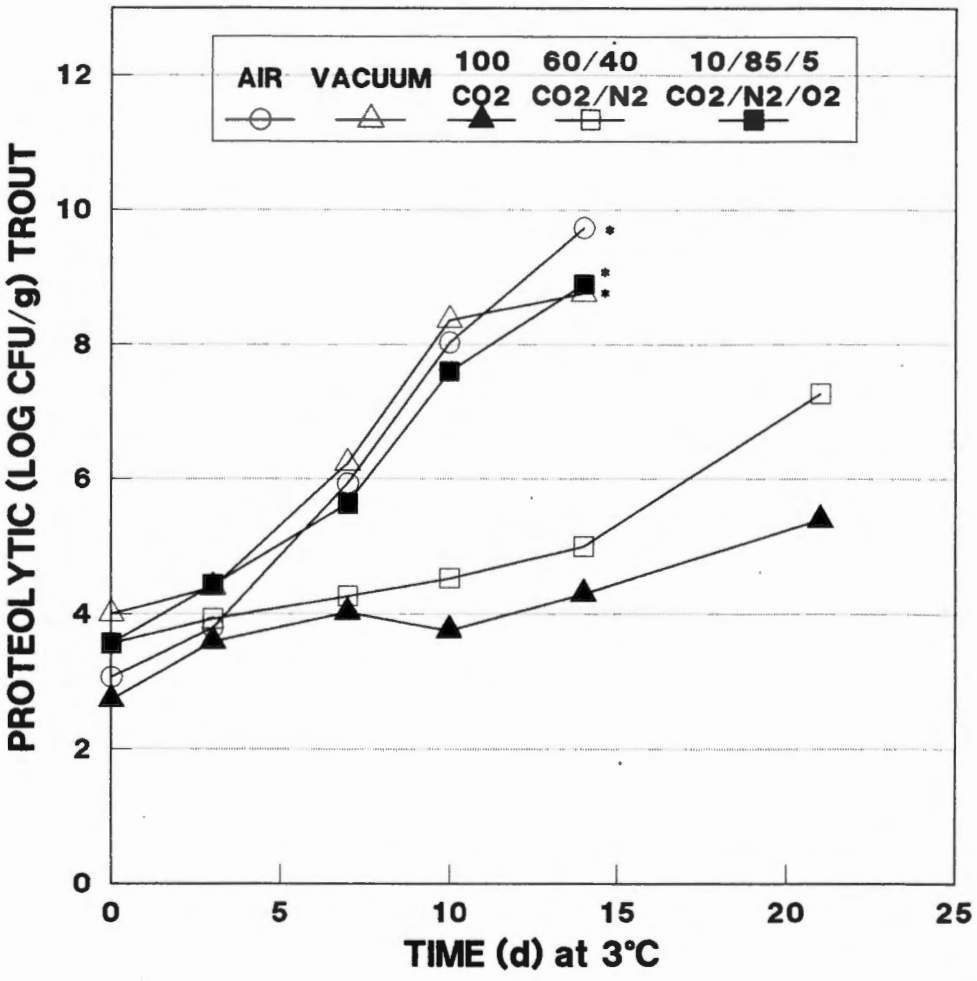

Figure 10. Proteolytic bacteria of trout fillets packaged under air, vacuum, and modified atmospheres at 3°C.
("*" point at which sampling was discontinued samples were considered spoiled.)

proteolytic bacterial count on the rainbow trout fillets initially ranged from log 2.5 to 4.5 CFU/g (Figures 9 and 10). At 3°C, the number of proteolytic bacteria in the trout packaged under air, vacuum, and 10/85/5 (carbon dioxide/nitrogen/oxygen) reached approximately log 8 CFU/g by day 10. The 100% CO^2 and 60/40 (carbon dioxide/nitrogen) proteolytic bacterial count was only log 7 CFU/g by day 21. At 10°C, the proteolytic bacterial count in the trout reached approximately log 8 CFU/g by day 6 in all treatments (Figures 9 and 10). Therefore, as temperature of storage increased, the inhibitory effect of CO_2 was diminished.

Lipolytic

At 3°C, lipolytic bacteria were significantly reduced in trout packaged in 100% CO_2 and 60/40 carbon dioxide/nitrogen (Appendixes A13). Lipolytic bacterial counts on the rainbow trout fillets initially ranged from log 2 to 4 CFU/g (Figures 11 and 12). During storage of trout samples held at 10°C, air, vacuum and 10/85/5 (carbon dioxide/nitrogen/oxygen) lipolytic counts reached approximately log 8 CFU/g by day 4. Lipolytic bacteria in trout packaged in 100% CO_2 and 60/40 (carbon dioxide/nitrogen) reached approximately log 8 CFU/g by day 6. At 3°C, the number of lipolytic bacteria in trout packaged under air, vacuum and 10/85/5 (carbon

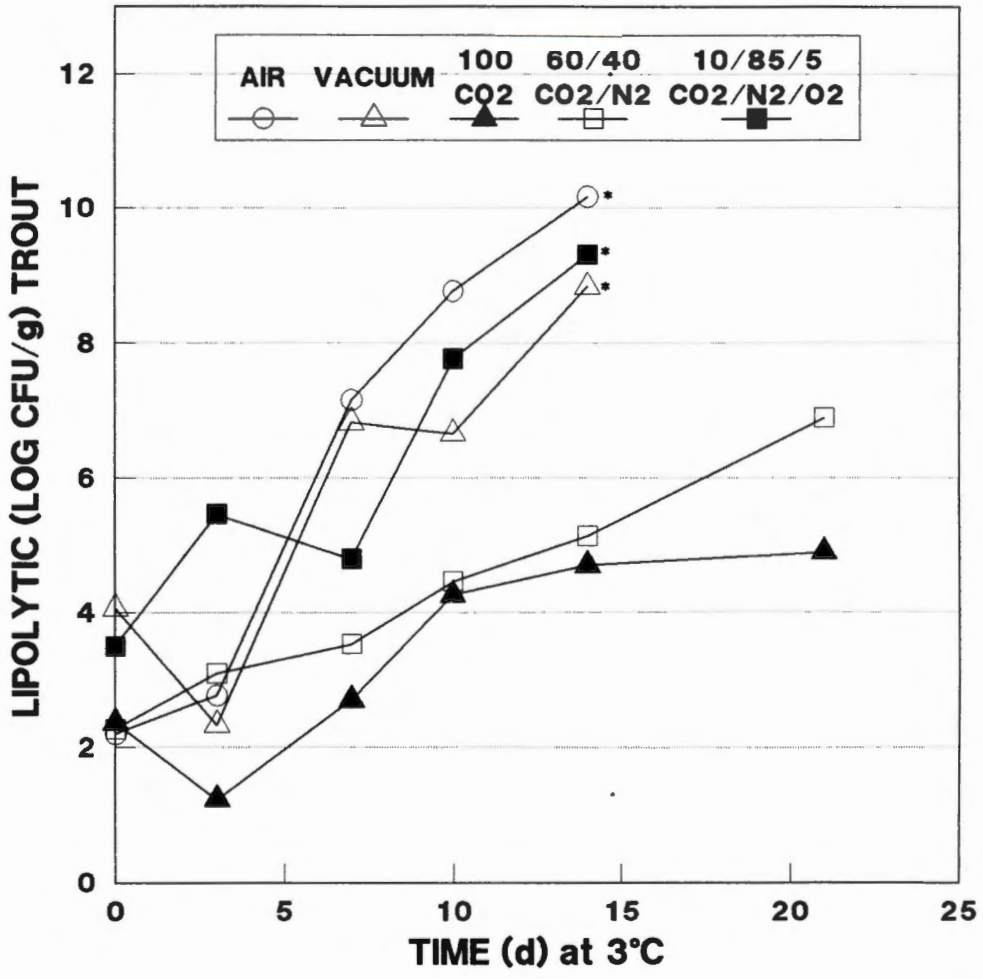

Figure 11. Lipolytic bacterial count of trout fillets packaged under air, vacuum, and modified atmospheres at 3°C.
("*" point at which sampling was discontinued, samples were considered spoiled.)

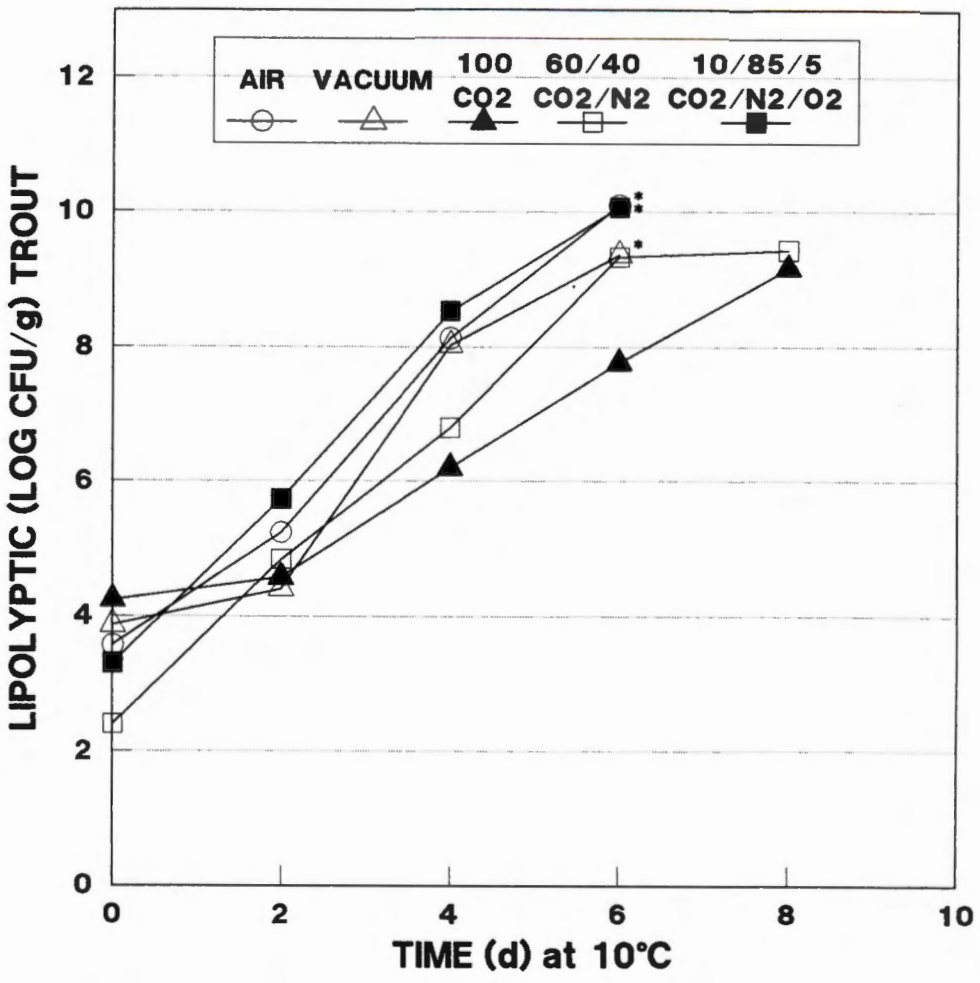

Figure 12. Lipolytic bacterial count of trout fillets packaged under air, vacuum, and modified atmospheres at 10°C.
("*" point at which sampling was discontinued, samples were considered spoiled.)

dioxide/nitrogen/oxygen) reached approximately log 8 CFU/g by day 14. By day 21, lipolytic organisms in trout packaged in 100% CO_2 and 60/40 (carbon dioxide/nitrogen) increased to approximately log 7 CFU/g (Figures 11 and 12).

Aerobic sporeformers

Atmosphere did not have a significant effect ($p > 0.05$) upon trout stored at 10°C or 3°C (Appendixes A16 and A17). The mean values for aerobic sporeformers on trout fillets packaged in 10/85/5 (carbon dioxide/nitrogen/oxygen), 60/40 (carbon dioxide/nitrogen), 100% CO_2, air, and vacuum packages were 3.0, 2.7, 2.3, 2.3, and 1.6 spores/g at 3°C and 12.3, 5.2, 7.7, 8.4, and 3.7 spores/g at 10°C, respectively (Appendixes A18 and A19). The aerobic sporeformer count on rainbow trout fillets initially ranged from approximately 3 to 16 spores/g at 10°C and approximately 1 to 4 spores/g at 3°C (Figures 13 and 14). The variations in aerobic sporeformers can be attributed to the time of catch of the rainbow trout and the season in which they were caught.

Anaerobic sporeformers

The effect of atmosphere on anaerobic sporeformers was found not to be significant ($p > 0.05$) at 10°C or 3°C (Appendixes A20 and A21). The mean anaerobic sporeformer

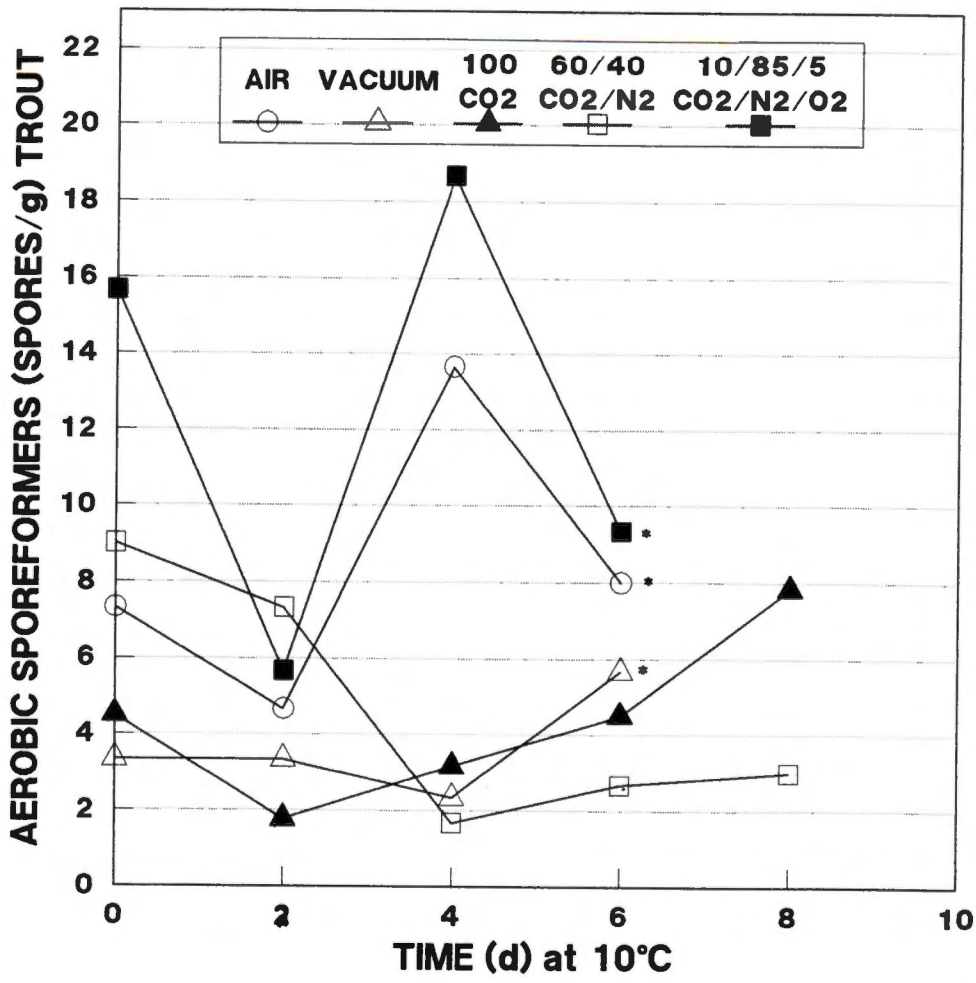

Figure 13. Aerobic Sporeformers in trout fillets packaged under air, vacuum, and modified atmospheres at 10°C.
("*" point at which sampling was discontinued, samples were considered spoiled.)

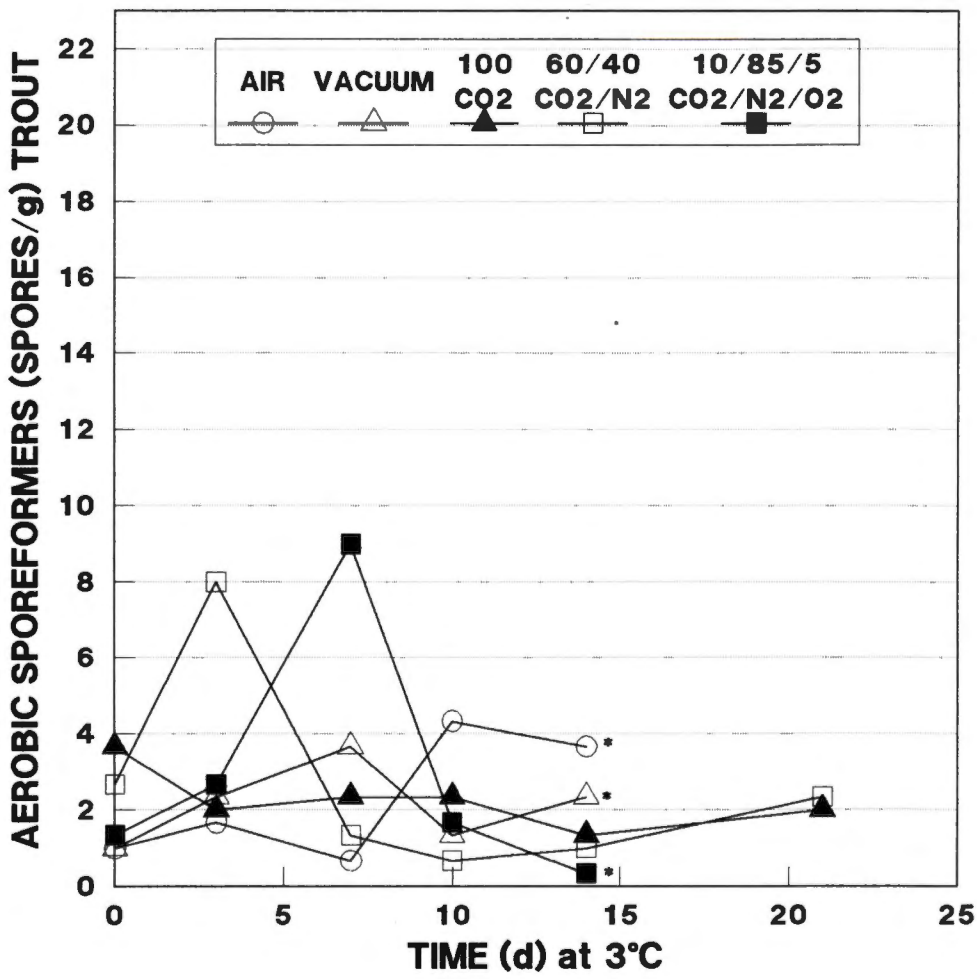

Figure 14. Aerobic Sporeformers in trout fillets packaged under air, vacuum, and modified atmospheres at 3°C.
("*" point at which sampling was discontinued, samples were considered spoiled.)

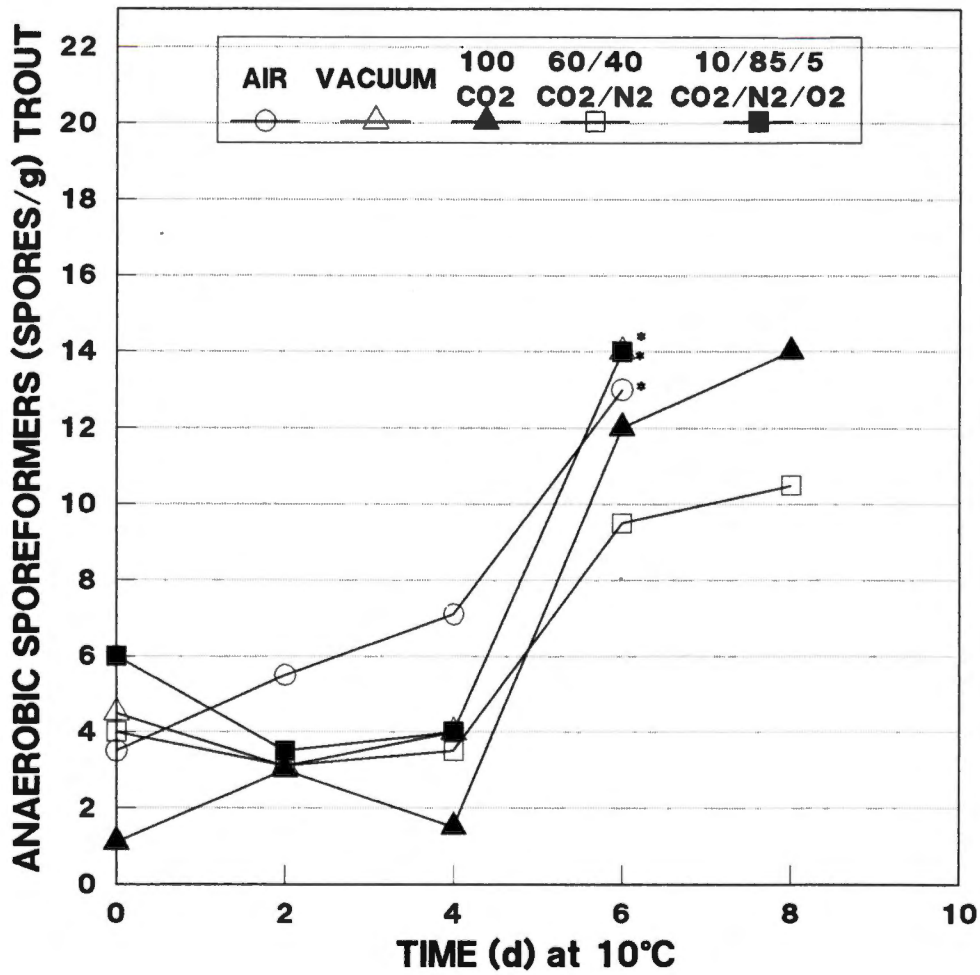

Figure 15. Anaerobic Sporeformer on trout fillets packaged under air, vacuum, and modified atmospheres at 10°C.
("*" point at which sampling was discontinued, samples were considered spoiled.)

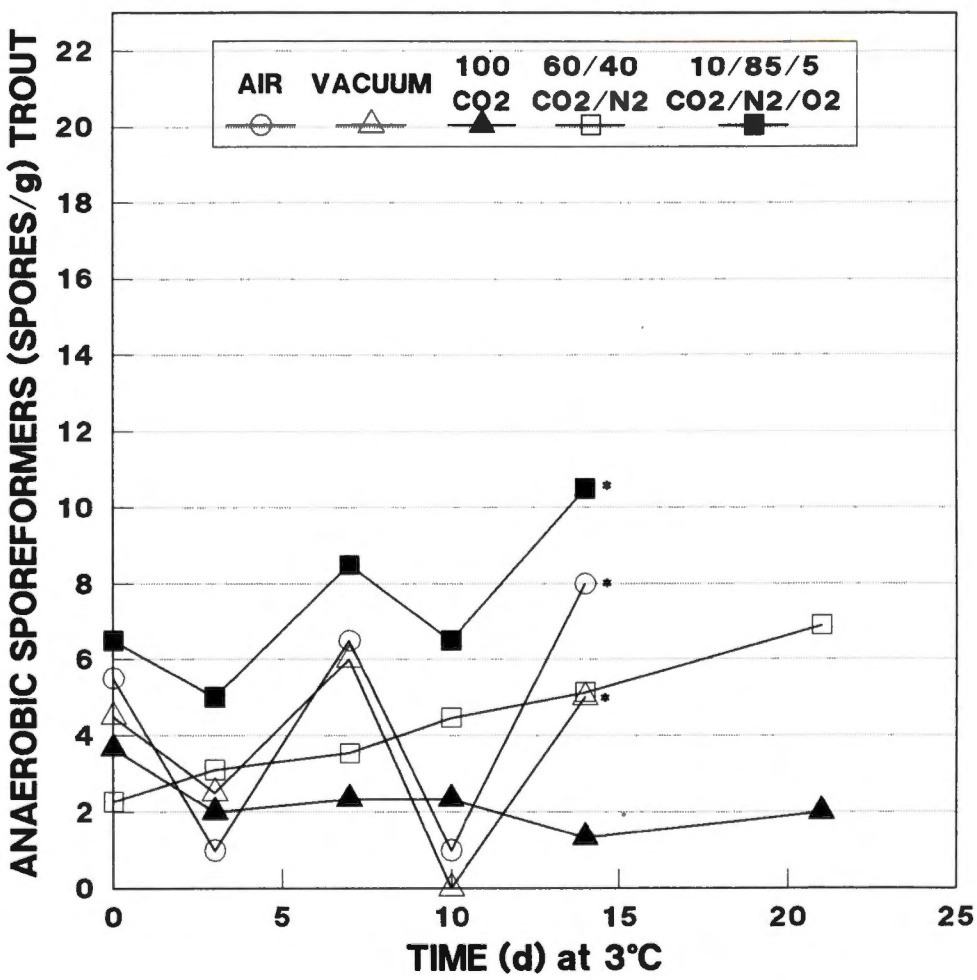

Figure 16. Anaerobic Sporeformer on trout fillets packaged under air, vacuum, and modified atmosphere at 3°C.
("*" point at which sampling was discontinued, samples were considered spoiled.)

counts increased significantly ($p < 0.05$) over time at 10°C; however, anaerobic spore counts did not increase significantly ($p > 0.05$) at 3°C. The initial values on rainbow trout stored at 3°C and 10°C were approximately 1 to 6 spores/g. Anaerobic spore counts increased up to 16 spores/g on fillets stored at 10°C (Figures 15 and 16). Anaerobic sporeformers on trout fillets were lower at 3°C than 10°C. Initial differences in spore counts may be attributed to random variation due to season and handling of trout.

Yeast and molds

The atmosphere of the package had a significant effect on the growth of yeast and mold in rainbow trout samples at 10°C and 3°C ($p < 0.05$) (Appendixes A22 and A23). The 100% CO_2 and 60/40 (carbon dioxide/nitrogen) had the lowest mean values with the yeast and mold counts initially ranged from approximately 25 CFU/g (Figures 17 and 18). At 3°C, the number of yeast and molds in trout packaged under air, vacuum and 10/85/5 (carbon dioxide/nitrogen/oxygen) increased to approximately to 125 CFU/g by day 14. Yeast and mold counts in trout packaged in 100% CO_2 and 60/40 (carbon dioxide/nitrogen) remained under 25 CFU/g up to day 21. At 10°C, yeast and mold counts reached approximately 285 CFU/g by day 6 in air and 10/85/5 (carbon dioxide/nitrogen/oxygen)

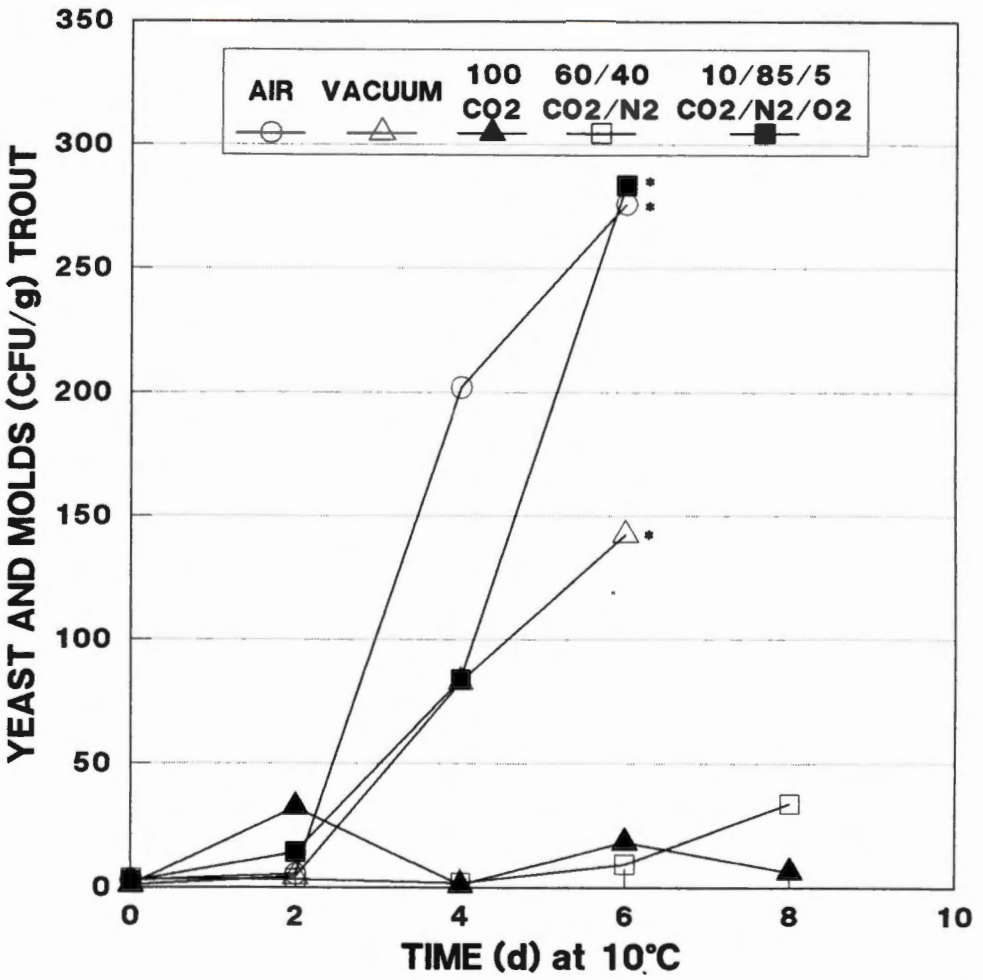

Figure 17. Yeast and mold count for trout fillets packaged under air, vacuum, and modified atmospheres at 10°C.
("*" point at which sampling was discontinued samples were considered spoiled.)

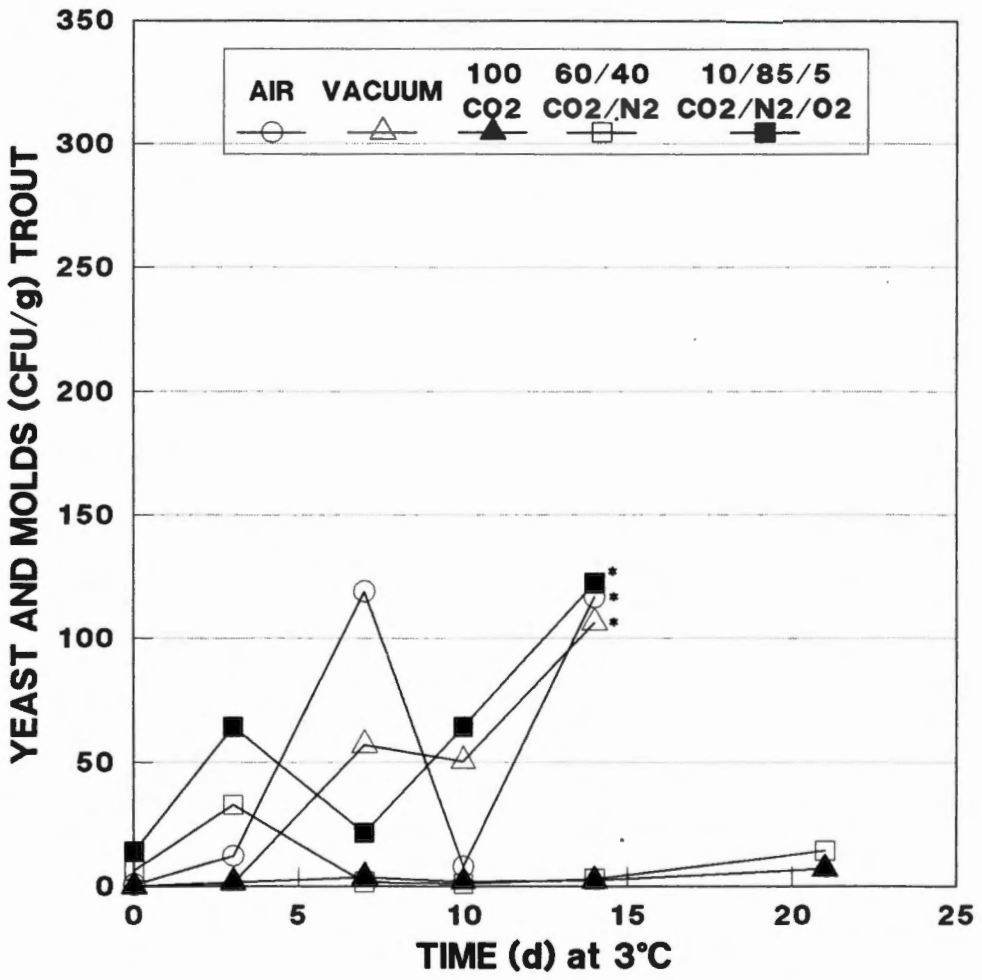

Figure 18. Yeast and mold count for trout fillets packaged under air, vacuum, and modified atmospheres at 3°C.
("*" point at which sampling was discontinued, samples were considered spoiled.)

packaged fillets. Fillets packaged in 60/40 (carbon dioxide/nitrogen) and 100% CO_2 had yeasts and mold counts of <40 CFU/g by day 8.

B. pH

The initial pH of rainbow trout fillets ranged between 6.4 and 6.6 and remained relatively constant for all samples (Figures 19 and 20). The 100% CO_2 atmosphere had a significantly lower pH at 3°C ($p < 0.05$) but did not have a significant effect upon pH at 10°C (Appendixes A24 and A25). The mean pH for trout packaged in air, vacuum, 100% CO_2, 60/40 (carbon dioxide/nitrogen) and 10/85/5 (carbon dioxide/nitrogen/oxygen) atmospheres at 10°C was 6.6, 6.5, 6.3, 6.5 and 6.5, respectively. At 3°C, the mean pH for trout packaged in air, vacuum, 100% CO_2, 60/40 (carbon dioxide/nitrogen) and 10/85/5 (carbon dioxide/nitrogen/oxygen) were 6.5, 6.5, 6.2, 6.3 and 6.4 at 3°C, respectively (Appendix A29). The results were related to the effect of CO_2 absorption on the surface of the trout. The pH data for trout fillets confirms the results observed by Barnett et al., (1987) for packaged rainbow trout. In our study, the probe was placed into the center of the trout fillet to determine pH. However, if pH had been determined from the surface of the trout, greater changes in values may have been observed.

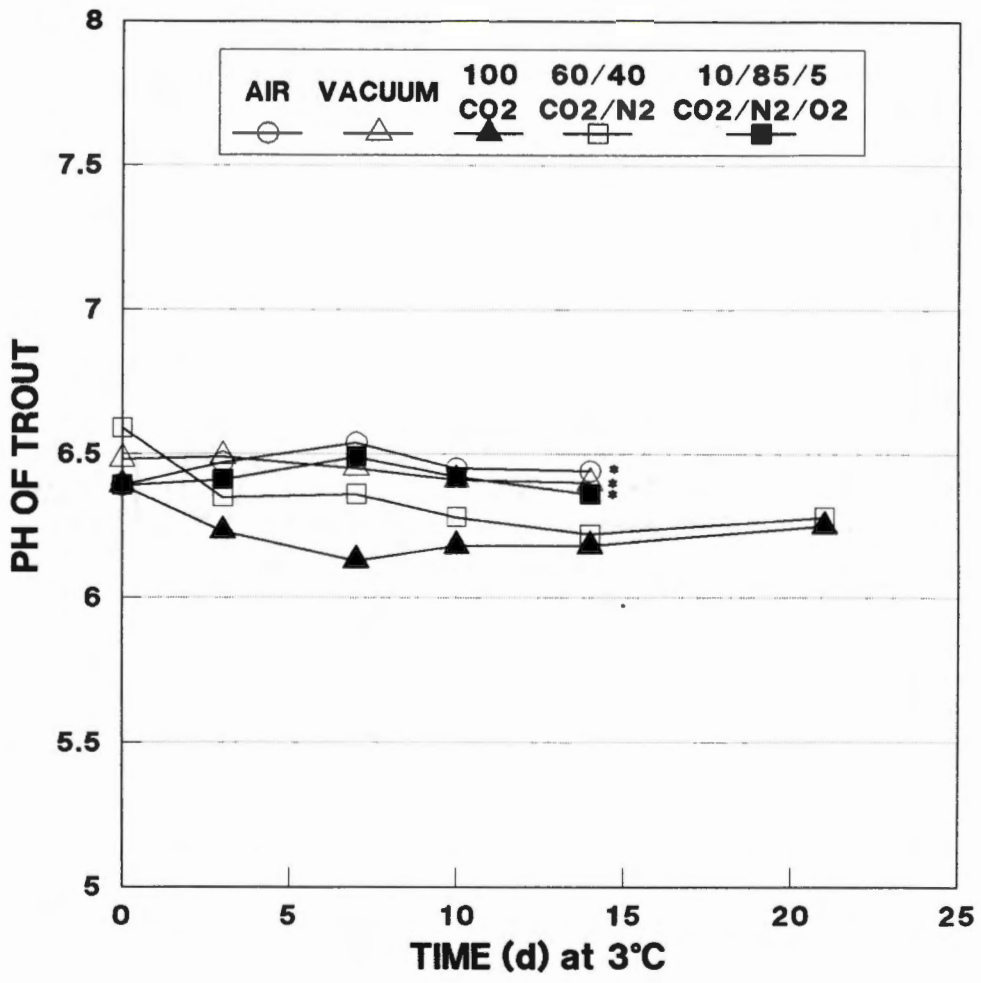

Figure 19. pH of trout fillets packaged under air, vacuum, and modified atmospheres at 3°C.
("*" point at which sampling was discontinued, samples were considered spoiled.)

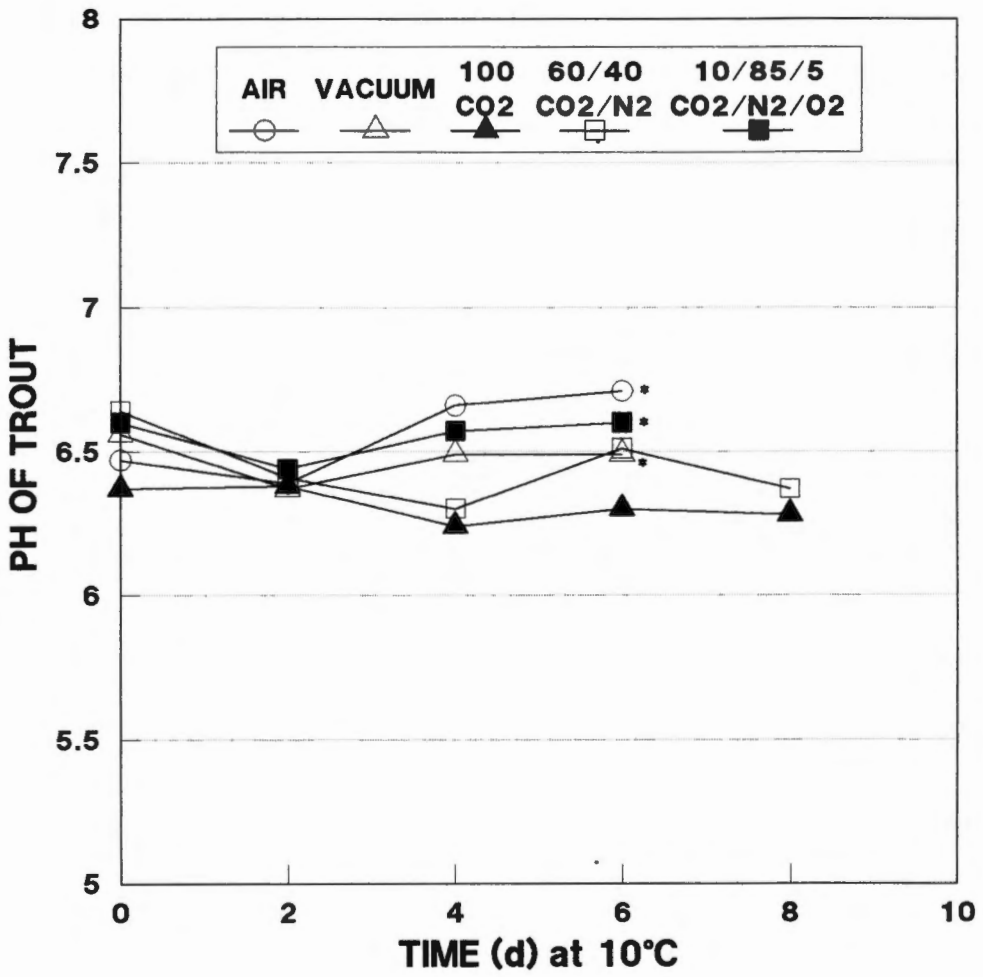

Figure 20. pH of trout fillets packaged under air, vacuum, and modified atmospheres at 10°C. ("*" point at which sampling was discontinued, samples were considered spoiled.)

C. Replication differences

There was no significant difference between replications of psychrotrophs plate count (PPC) or anaerobic sporeformers at 10°C ($p > 0.05$) (Appendixes A26 and A27). In addition, there were no a significant differences observed among replications in yeast and mold counts stored at the 10°C storage temperature ($p > 0.05$) (Appendix A26). At 3°C, there were a significant differences among replications for lipolytic counts and pH ($p < 0.05$) (Appendixes A28 and A29). Mean separation tables at 3°C for pH showed that replications one and two were significantly different from the third replication ($p < 0.05$) (Appendix A30). Mean separation tables at 10°C in pH observed that replication one was significantly different from two and three ($p < 0.05$) (Appendix A30). Differences in replications may be related to the seasonal changes in the microbial load, time of catch, or often using a different group of fish for each replication (Connell, 1980).

D. Headspace analysis

The composition of the initial atmosphere in air packaged fillets of rainbow trout consisted of approximately 21.4% O_2, 0.5% CO_2, and the balance N_2. At 10°C, a decline in O_2 was observed over storage time along with an increase in CO_2

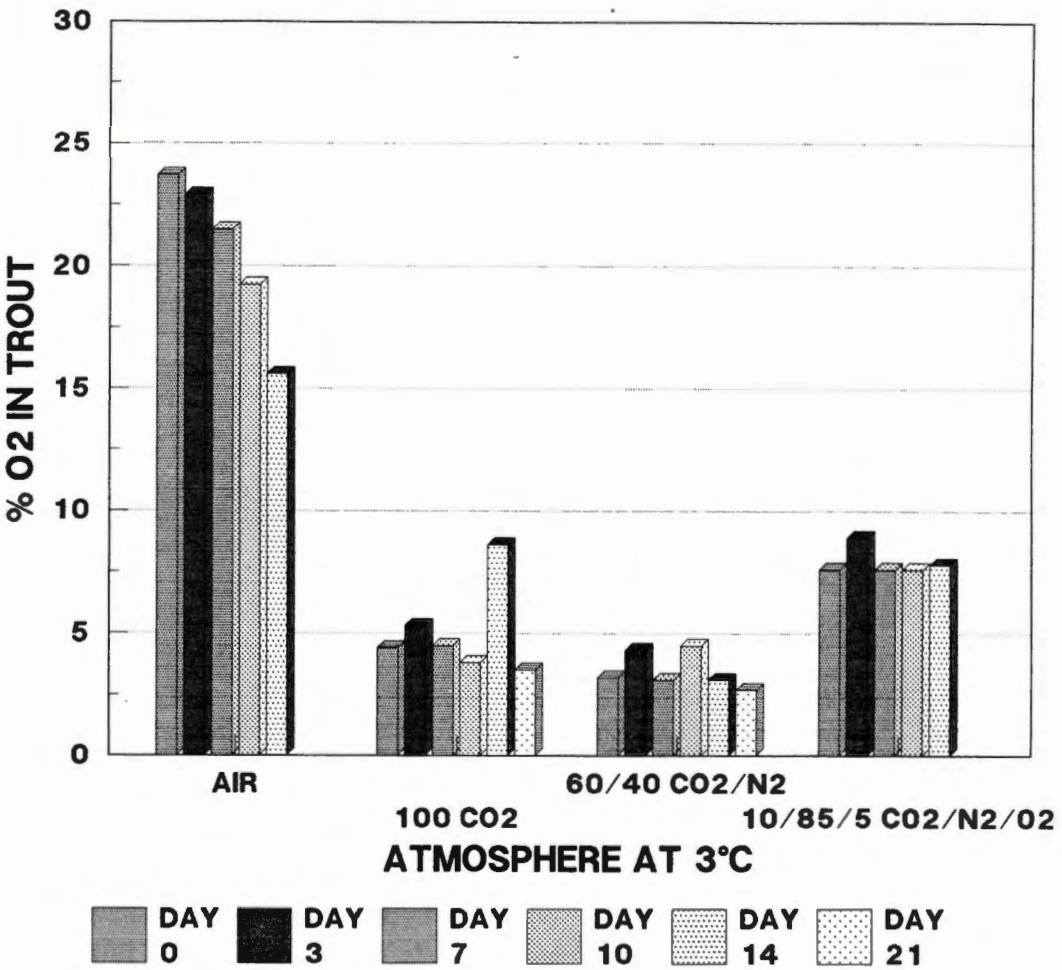

Figure 21. Changes in O_2 composition (%) of modified atmosphere for rainbow trout fillets packaged under air, vacuum, and modified atmospheres at 3°C.

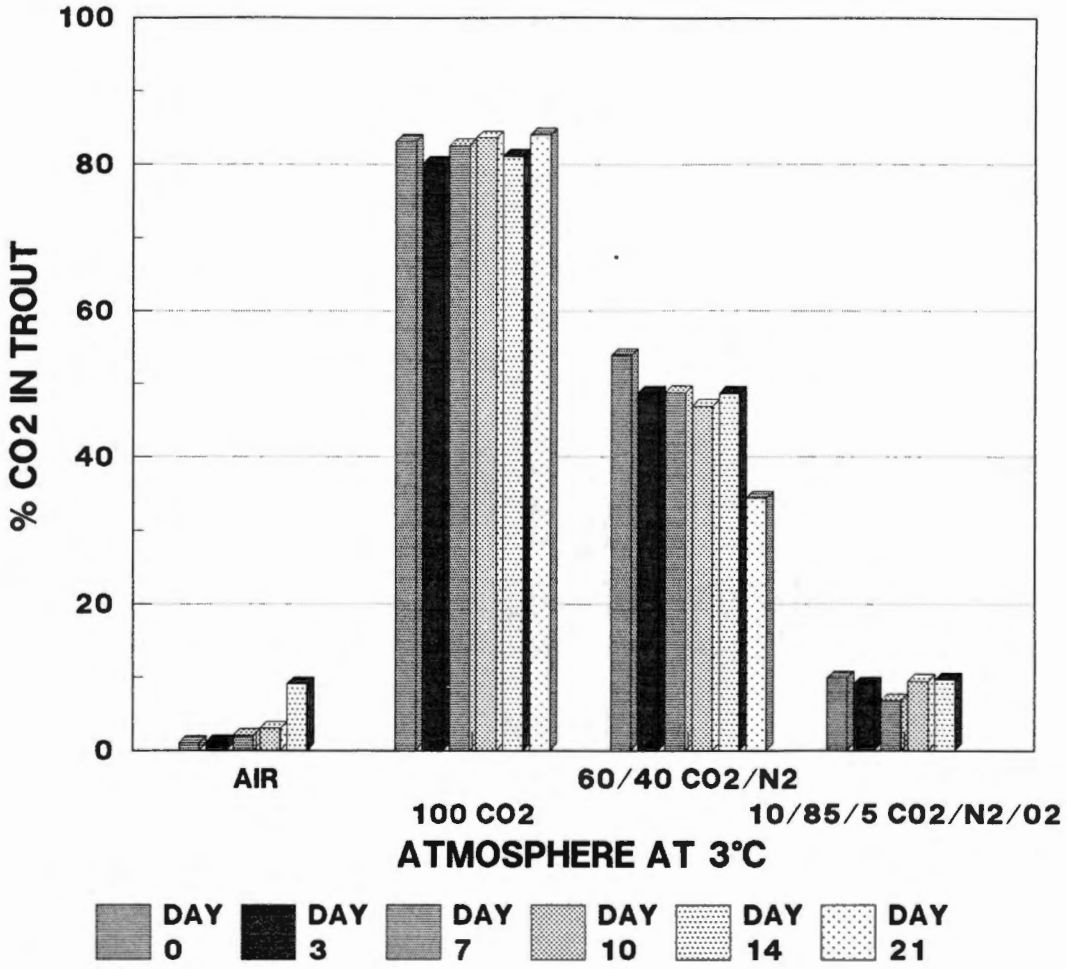

Figure 22. Changes in CO_2 composition (%) of modified atmosphere for rainbow trout fillets packaged under air, vacuum, and modified atmospheres at 3°C.

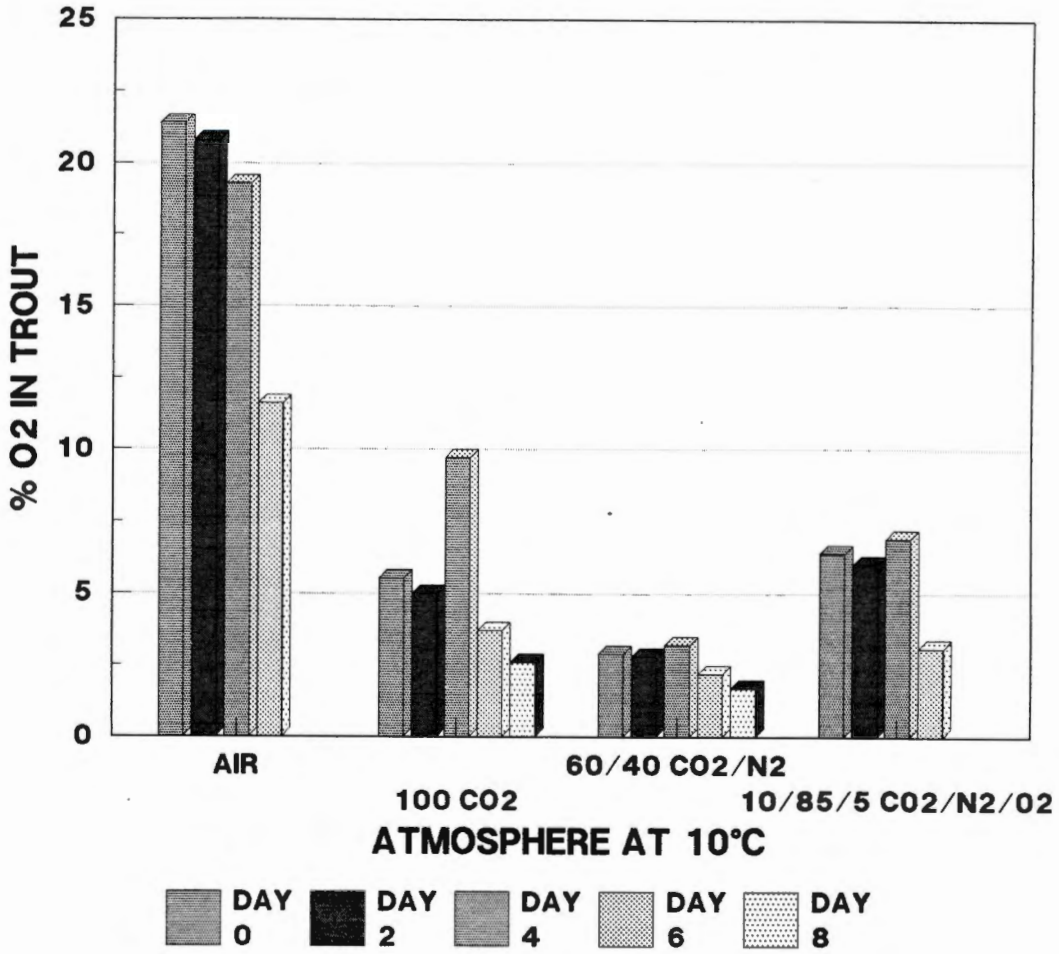

Figure 23. Changes in O_2 composition (%) of modified atmosphere rainbow trout fillets packaged under air, vacuum, and modified atmospheres at 10°C.

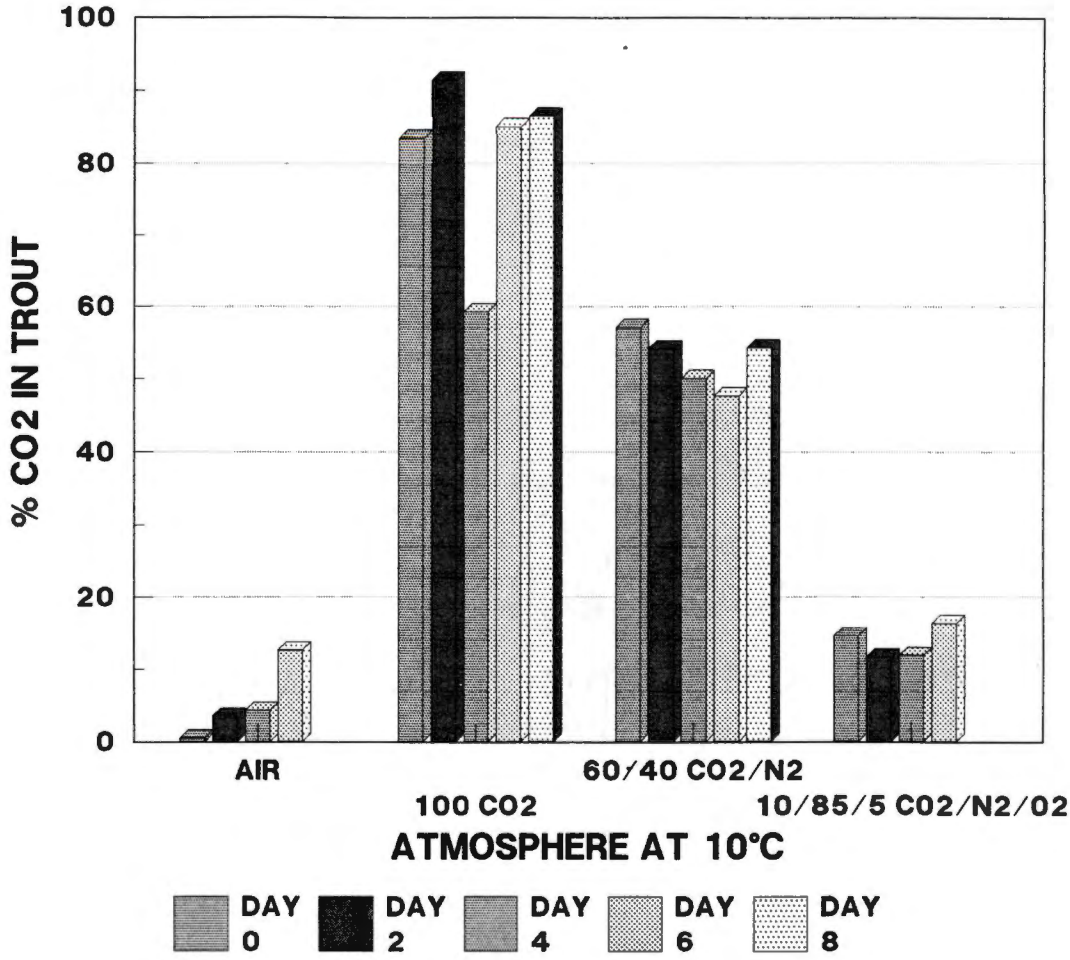

Figure 24. Changes in CO_2 composition (%) of modified atmosphere rainbow trout fillets packaged under air, vacuum, and modified atmospheres at 10°C.

(Figures 23 and 24). Similar trends in atmosphere of fillets packaged in air stored at 3°C (Figures 21 and 22) were also observed. However, the gas composition was approximately the same concentrations at day 14 at 3°C as day 7 at 10°C. Trout stored in air, vacuum, and 10/85/5 (carbon dioxide/nitrogen/oxygen) at 3°C had approximately 8 days longer in shelf life than trout stored at 10°C. The increase in CO_2 and decrease in O_2 resulted due to microbial action and chemical reactions which utilized oxygen and produce carbon dioxide.

The composition of the 100% CO_2 gas mixture initially measured in the packages of rainbow trout fillets was 83.5% CO_2, and 5.5% O_2 and balance of N_2. Levels of CO_2 were maintained over time at 10°C reaching 86.6% by day 8 and 84% by day 21 at 3°C with O_2 levels remaining relatively low (Figures 21-24). The slight decrease in O_2 may be attributed to oxygen in the fish flesh and the oxygen from the packaging tray. Initial levels of CO_2 were not 100% in the packages sealed with 100% CO_2 gas. Slight amounts of oxygen may have also entered the package due to improper sealing of the packaged fillets, or leakage at the gas tanks. Any one of these factors could have caused the initial reading of the 100% CO_2 to fall into the 85% range.

The composition of the 60/40 (carbon dioxide/nitrogen)

gas mixture initially measured in the packages of fillets consisted of 57.3% CO_2 and 2.9% O_2 and the balance N_2. Levels of CO_2 decreased slightly over time reaching 54.4% by day 8 at 10°C and 84.0% by day 21 at 3°C with levels of O_2 slightly fluctuating over storage time (Figures 21 and 24). Gas composition changed very little with storage.

The composition of the 10/85/5 (carbon dioxide/nitrogen/oxygen) gas mixture initially measured in the packages of trout fillets consisted of 14.6% CO_2 and 6.4% O_2 and the balance of N_2. Levels of CO_2 were relatively lower at 3°C than levels of CO_2 at 10°C. The level of CO_2 reached 9.7% by day 14 at 3°C and 7.1% by day 6 at 10°C (Figures 21-24). The spoilage level was extended 8 days in fillets packaged in air, vacuum, and 10/85/5 (carbon dioxide/nitrogen/oxygen) at 3°C. At 3°C, the spoilage level was extended 13 days for fillets packaged in 100% CO_2, and 60/40 (carbon dioxide/nitrogen). Headspace gas analysis of the vacuum packaged fillets was not performed because a vacuum was maintained throughout storage with no indications of leakage to the fish product.

E. Temperature control

The temperature variation inside the incubators remained relatively constant (3° ± 0.5°C, 10° ± 0.5°C). When the door was

opened to remove the fillet samples, the air temperature inside the incubator increased by 5°C and returned to baseline (3°C or 10°C) within 2 minutes. Therefore, a strict temperature adherence was maintained on the fillets.

CHAPTER V

CONCLUSION

High concentrations of CO_2 without the presence of oxygen used in a modified atmosphere (100% CO_2 and 60/40, carbon dioxide/nitrogen) effectively extended the shelf life of fresh rainbow trout fillets. The presence of oxygen encouraged the growth of aerobic sporeformers, yeast and molds, coliforms, lipolytic, proteolytic, aerobic plate count, and psychrotrophic plate counts on the packaged fillets. Rainbow trout stored at 3°C had lower bacteria counts, sporeformers, and yeast and mold growth than did fillets packaged at 10°C. *Listeria* and *Salmonella* were not detected in fillets. In addition, fillets stored at 3°C had a better overall quality in comparison with fillets stored at 10°C. The overall quality of the fillets was based upon appearance, odor, color, and texture. Fillets stored at 10°C deteriorated at a faster rate than fillets stored at 3°C. Therefore, the rate of microbial growth was controlled and chemical and organoleptic changes were minimized by modified atmosphere packaging and strict temperature control.

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APPENDIXES

Table A1. Anova values for aerobic plate count on rainbow trout stored at 3°C.

Source	DF	Mean Square	Prob > F
ATM	4	21.1365	0.0001
DAY	4	38.5940	0.0001
REP	2	0.1308	0.7723
ATM*DAY	16	3.1282	0.0001
ERROR	48	0.5034	

Table A2. Anova values for aerobic plate count on rainbow trout stored at 10°C.

Source	DF	Mean Square	Prob > F
ATM	4	5.7176	0.0003
DAY	3	107.2366	0.0001
REP	2	5.6831	0.0032
ATM*DAY	12	1.3777	0.1267
ERROR	38	0.8496	

Table A3. Mean separation values between atmospheres for microbial counts (log_{10} CFU/g) in rainbow trout stored at 3°C.

ATM	APC	PSY	COL	LAC	PROT	LIPO	YM
AIR	6.8^{a}	6.4^{a}	4.8^{a}	5.1^{ab}	6.1^{a}	6.2^{a}	66.6^{a}
VAC	6.4^{a}	6.2^{a}	4.7^{a}	5.0^{ab}	6.4^{a}	5.7^{a}	43.1^{a}
100	3.5^{b}	3.9^{b}	2.4^{b}	4.2^{b}	3.7^{b}	2.9^{b}	1.9^{b}
60/40	4.6^{b}	4.3^{b}	3.2^{b}	3.9^{b}	4.3^{b}	3.7^{b}	9.0^{b}
10/85/5	6.7^{a}	6.5^{a}	5.2^{a}	5.6^{a}	6.0^{a}	6.2^{a}	57.3^{a}

Within each column, means with the same letter are not significantly different ($p \leq 0.05$) using the Student-Newman Keuls mean separation test.

Table A4. Mean separation values between atmospheres for microbial counts (log_{10} CFU/g) in rainbow trout stored at 10°C.

ATM	APC	PSY	COL	LAC	PROT	LIPO	YM
AIR	7.5^{a}	7.1^{ab}	5.8^{ab}	6.7^{ab}	7.5^{a}	6.8^{a}	99.6^{a}
VAC	7.1^{ab}	6.7^{bc}	5.3^{b}	6.6^{ab}	7.0^{a}	5.8^{a}	57.8^{b}
100	6.4^{bc}	6.1^{c}	4.7^{b}	5.5^{b}	5.9^{b}	5.7^{a}	13.3^{c}
60/40	6.1^{c}	6.3^{c}	4.7^{b}	6.1^{ab}	6.5^{ab}	5.8^{a}	4.6^{d}
10/85/5	7.7^{a}	7.5^{a}	6.6^{a}	7.2^{a}	7.6^{a}	6.9^{a}	96.1^{a}

Within each column, means with the same letter are not significantly different ($p \leq 0.05$) using the Student-Newman Keuls mean separation test.

Table A5. Anova values for psychrotrophic plate count on rainbow trout stored at 3°C.

Source	DF	Mean Square	Prob > F
ATM	4	24.3614	0.0001
DAY	4	39.4938	0.0001
REP	2	0.0412	0.9605
ATM*DAY	16	3.3485	0.0007
ERROR	48	1.0223	

Table A6. Anova values for psychrotrophic plate count on rainbow trout stored at 10°C.

Source	DF	Mean Square	Prob > F
ATM	4	4.0076	0.0001
DAY	3	100.1201	0.0001
REP	2	0.0967	0.1479
ATM*DAY	12	0.4004	0.6176
ERROR	38	0.4810	

Table A7. Anova values for coliforms on rainbow trout stored at 3°C.

Source	DF	Mean Square	Prob > F
ATM	4	21.3466	0.0001
DAY	4	23.4523	0.0001
REP	2	0.3721	0.8422
ATM*DAY	16	4.3956	0.0297
ERROR	48	2.1594	

Table A8. Anova values for coliforms on rainbow trout stored at 10°C.

Source	DF	Mean Square	Prob > F
ATM	4	7.4120	0.0040
DAY	3	132.6831	0.0001
REP	2	23.0765	0.0001
ATM*DAY	12	2.5174	0.1455
ERROR	38	1.6126	

Table A9. Anova values for lactics of rainbow trout stored at 3°C.

Source	DF	Mean Square	Prob > F
ATM	4	7.0498	0.0045
DAY	4	35.2631	0.0001
REP	2	1.5292	0.3970
ATM*DAY	16	1.3580	0.6400
ERROR	48	1.6235	

Table A10. Anova values for lactics on rainbow trout stored at 10°C.

Source	DF	Mean Square	Prob > F
ATM	4	5.2922	0.0184
DAY	3	94.2742	0.0001
REP	2	7.2821	0.0156
ATM*DAY	12	2.0099	0.2672
ERROR	38	1.5647	

Table A11. Anova values for proteolytic on rainbow trout stored at 3°C.

Source	DF	Mean Square	Prob > F
ATM	4	22.4354	0.0001
DAY	4	40.4181	0.0001
REP	2	0.0292	0.9646
ATM*DAY	16	3.5469	0.0001
ERROR	48	0.8098	

Table A12. Anova values for proteolytic on rainbow trout stored at 10°C.

Source	DF	Mean Square	Prob > F
ATM	4	5.4205	0.0009
DAY	3	87.7175	0.0001
REP	2	11.8906	0.0001
ATM*DAY	12	0.5590	0.0001
ERROR	38	0.9222	

Table A13. Anova values for lipolytic on rainbow trout stored at 3°C.

Source	DF	Mean Square	Prob > F
ATM	4	35.3303	0.0001
DAY	4	62.9651	0.0001
REP	2	10.8172	0.0317
ATM*DAY	16	4.9178	0.0821
ERROR	48	2.9130	

Table A14. Anova values for lipolytic on rainbow trout stored at 10°C.

Source	DF	Mean Square	Prob > F
ATM	4	4.0035	0.1396
DAY	3	97.6373	0.0001
REP	2	7.0286	0.0499
ATM*DAY	12	1.8253	0.0607
ERROR	38	2.1649	

Table A15. Mean separation between replications for sporeformers (spores/g) in rainbow trout stored at 3°C.

REP	AESP	ANSP
1	3.6[a]	
2	1.8[a]	4.2[a]
3	1.2[a]	6.9[a]

Within each column, means with the same letter are not significantly different ($p \leq 0.05$) using the Student-Newman Keuls mean separation test.

Table A16. Anova values for aerobic sporeformers on rainbow trout stored at 3°C.

Source	DF	Mean Square	Prob > F
ATM	4	4.2466	0.8817
DAY	4	8.5133	0.6747
REP	2	27.6933	0.1600
ATM*DAY	16	16.5833	0.3476
ERROR	48	14.5405	

Table A17. Anova values for aerobic sporeformers on rainbow trout stored at 10°C.

Source	DF	Mean Square	Prob > F
ATM	4	133.0666	0.1679
DAY	3	52.9944	0.5691
REP	2	1815.0000	0.0001
ATM*REP	12	43.9111	0.8564
ERROR	38	77.8070	

Table A18. Mean separation values between atmospheres for sporeformers (spores/g) in rainbow trout stored at 3°C.

ATM	AESP	ANSP
AIR	2.3[a]	4.4[a]
VAC	1.6[a]	3.6[a]
100	2.3[a]	3.9[a]
60/40	2.7[a]	8.0[a]
10/85/5	3.0[a]	7.4[a]

Within each column, means with the same letter are not significantly different ($p \leq 0.05$) using the Student-Newman Keuls mean separation test.

Table A19. Mean separation values between atmospheres for sporeformers (spores/g) in rainbow trout stored at 10°C.

ATM	AESP	ANSP
AIR	8.4[a]	7.3[a]
VAC	3.7[a]	6.8[a]
100	7.7[a]	4.4[a]
60/40	5.2[a]	5.0[a]
10/85/5	12.3[a]	6.9[a]

Within each column, means with the same letter are not significantly different ($p \leq 0.05$) using the Student-Newman Keuls mean separation test.

Table A20. Anova values for anaerobic sporeformers on rainbow trout stored at 3°C.

Source	DF	Mean Square	Prob > F
ATM	4	43.0800	0.2527
DAY	4	43.4300	0.2491
REP	2	74.4200	0.1284
ATM*DAY	16	22.8675	0.7091
ERROR	24	30.0033	

Table A21. Anova values for anaerobic sporeformers on rainbow trout stored at 10°C.

Source	DF	Mean Square	Prob > F
ATM	4	14.5115	0.5763
DAY	3	187.6141	0.0003
REP	2	1.8075	0.9126
ATM*DAY	12	5.1191	0.9903
ERROR	22	19.6747	

Table A22. Anova values for yeast and mold on rainbow trout at 3°C.

Source	DF	Mean Square	Prob > F
ATM	4	12487.8466	0.0004
DAY	4	10751.1800	0.0012
REP	2	1261.2933	0.5393
ATM*DAY	16	3330.9633	0.0910
ERROR	48	2016.5017	

Table A23. Anova values for yeast and mold on rainbow trout stored at 10°C.

Source	DF	Mean Square	Prob > F
ATM	4	2701.7875	0.0001
DAY	3	44105.2917	0.0001
REP	1	60.0250	0.6785
ATM*DAY	12	9497.1875	0.0001
ERROR	19	338.8145	

Table A24. Anova values for pH on rainbow trout stored at 3°C.

Source	DF	Mean Square	Prob > F
ATM	4	0.1455	0.0001
DAY	4	0.0338	0.0001
REP	2	0.0573	0.0001
ATM*DAY	16	0.0219	0.0001
ERROR	48	0.0047	

Table A25. Anova values for pH on rainbow trout stored at 10°C.

Source	DF	Mean Square	Prob > F
ATM	4	0.1044	0.1974
DAY	3	0.0451	0.5661
REP	2	0.6485	0.0004
ATM*DAY	12	0.0207	0.9814
ERROR	38	0.0657	

Table A26. Mean separation values between replications for bacterial counts (log_{10} CFU/g) in rainbow trout stored at 10°C.

REP	APC	PSY	COL	LAC	PROT	LIPO	YM
1	7.6^a	7.0^a	6.6^a	7.1^a	7.7^a	6.8^a	
2	6.7^b	6.6^a	4.8^b	6.0^b	6.7^b	6.1^b	57.5^a
3	6.6^b	6.6^a	4.8^b	6.1^b	6.2^b	5.7^b	59.9^a

Within each column, means with the same letter are not significantly different ($p \leq 0.05$) using the Student-Newman Keuls mean separation test.

Table A27. Mean separation values between replications for sporeformers (spores/g) in rainbow trout stored at 10°C.

REP	AESP	ANSP
1	18.5[a]	7.3[a]
2	2.0[b]	6.4[a]
3	2.0[b]	5.8[a]

Within each column, means with the same letter are not significantly different ($p \leq 0.05$) using the Student-Newman Keuls mean separation test.

Table A28. Mean separation values between replications for microbial counts (log_{10} CFU/g) in rainbow trout stored at 3°C.

Rep	APC	PSY	COL	LAC	PROT	LIPO	YM
1	5.7[a]	5.5[a]	4.2[a]	4.9[a]	5.3[a]	4.3[a]	43.4[a]
2	5.8[a]	5.4[a]	4.0[a]	4.9[a]	5.3[a]	5.0[a]	29.5[a]
3	5.9[a]	5.5[a]	3.9[a]	4.5[a]	5.3[a]	5.6[a]	33.8[a]

Within each column, means with the same letter are not significantly different ($p \leq 0.05$) using the Student-Newman Keuls mean separation test.

Table A29. Mean separation values between replications for pH in rainbow trout stored at 3°C and 10°C.

REP	pH (3°C)	pH (10°C)
1	6.3[b]	6.7[a]
2	6.4[b]	6.4[b]
3	6.4[a]	6.3[b]

Within each column, means with the same letter are not significantly different ($p \leq 0.05$) using the Student-Newman Keuls mean separation test.

Table A30. Mean separation values between atmospheres for pH in rainbow trout stored at 3°C and 10°C.

ATM	pH (3°C)	pH (10°C)
AIR	6.5[a]	6.6[a]
VAC	6.5[a]	6.5[a]
100	6.2[c]	6.3[a]
60/40	6.3[b]	6.5[a]
10/85/5	6.4[a]	6.5[a]

Within each column, means with the same letter are not significantly different ($p \leq 0.05$) using the Student-Newman Keuls mean separation test.

VITA

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