INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

FORMULATION, SHELF-LIFE AND SAFETY STUDIES ON VALUE-ADDED TROUT PRODUCTS PACKAGED UNDER MODIFIED ATMOSPHERES

By:

Jiun Ni Liu

Department of Food Science and Agricultural Chemistry
Macdonald Campus, McGill University
Montreal, Quebec

A Thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment for the requirements for the degree of Master of Science

May 2000

ABSTRACT

FORMULATION, SHELF-LIFE AND SAFETY STUDIES ON VALUE-ADDED TROUT PRODUCTS PACKAGED UNDER MODIFIED ATMOSPHERES

Value-added trout burgers and trout wontons were prepared from minced trout trimmings, oats, dried onion flakes, white pepper, cayenne pepper, garlic powder, soy sauce, spice mix and eggs. The trout burgers were coated with crushed cornflakes and then partially cooked (2 min at 140°C) or fully cooked (5 min at 140°C) in hot oil. Similar fillings were used to prepare trout wontons by wrapping the mixture with Chinese wonton dough and 2 products were made: raw wonton (uncooked) and fried wonton (fried for 8 min at 140°C).

Storage trials were performed on raw and fried trout wontons (a_w 0.98-0.95, pH 6.5), and partially and fully cooked trout burgers (a_w 0.97-0.96, pH 6.4). Products were packaged in air and under various modified atmospheres (MAP), and stored at 4 and 12°C. A microbiological shelf-life of >28 days was possible for the fully cooked trout burgers and fried wontons stored at 4°C. In general, the microbiological shelf-life preceded the sensory shelf-life.

Subsequent challenge studies were done to address the safety concerns associated with MAP food. All products were inoculated with 10^2 CFU/g of *Listeria monocytogenes* and 10^2 spores/g of *Clostridium botulinum* type E spores. Gas packaging with 80% CO_2 (balance N_2) inhibited the growth of *L. monocytogenes* in products stored at 4°C. However, counts of *L. monocytogenes* increased in all other packaging conditions. In challenge studies with *C. botulinum* type E, toxin was not detected in any products after 28-60 days.

RÉSUMÉ

ÉTUDES DE FORMULATION, DE CONSERVATION ET D'INNOCUITÉ ALIMENTAIRE DE PRODUITS DE TRUITE DE VALEUR AJOUTÉE

Des burgers et des wontons de truite ont été préparés à partir d'émincés de truite, de flocons d'avoine, de flocons d'oignons séchés, de poivre blanc, de poivre de Cayenne, de poudre d'ail, de sauce soja, de mélanges d'épices et d'oeufs. Les burgers de truite, enrobés de flocons de mais écrasés, ont été partiellement cuits (2 minutes à 140°C) ou complètement cuits (5 minutes à 140°C) dans l'huile. Une farce similaire a été utilisée pour la préparation des wontons de truite. Ces derniers ont été réalisés en enrobant le mélange de poisson avec de la pâte chinoise à wontons. Deux produits ont été faits, soit des wontons crus (non-cuits) et des wontons frits (frits pendant 8 minutes à 140°C).

Des études de conservation ont été réalisées sur les wontons crus et frits (a_w 0.98-0.95, pH 6.5) et sur les burgers partiellement et complètement cuits (a_w 0.97-0.96, pH 6.4). Les produits, emballés sous air et sous différentes atmosphères modifiées, ont été gardés à 4 et 12°C. Une durée de vie microbiologique supérieure à 28 jours a été possible pour les burgers complètement cuits et les wontons frits gardés à 4°C. En général, la durée de vie microbiologique était plus courte que la durée de vie sensorielle.

Subséquemment, des études de cas ont été menées afin d'adresser l'inquiétude sur la sécurité alimentaire concernant les produits emballés sous atmosphères modifiées. Pour se faire, tous les produits ont été inoculés avec 10^2 UFC/g de *Listeria monocytogenes* et 10^2 spores/g de *Clostridium botulinum* de type E. La croissance de la *L. monocytogenes* a été inhibée par un emballage de 80% CO_2 (balance N_2) lorsque les produits étaient gardés à 4°C. Par contre, les comptes de *L. monocytogenes* ont augmenté dans toutes les autres conditions d'emballage. Aucune toxine botulinique n'a été détectée après 28-60 jours, dans aucun produit, lors d'études de cas menées avec le *C. botulinum* de type E.

ACKNOWLEDGMENTS

I would like to express my most sincere thanks to my supervisor, Dr. J.P. Smith for his knowledge, guidance and patience throughout this study.

I would also like to thank Ms. Ilsemarie Tarte and Ms. Caroline Simard for their assistance in carrying out the laboratory experiments. Special thanks to Ms. Isabelle Dufresne who helped me all along with my project. My appreciation is also extended to everyone else in the research group, including Ms. Daphne Phillips, Mr. Sameer Al-Zenki, Mr. Anis El-Khoury, Mr. Galen Bagdan, Mr. Wassim El-Khoury and Mr. John Koukoutsis for their friendship, assistance and support.

Additional thanks to Ms. Isabelle Dufresne for translating the abstract.

I would like to thank Dr. John Austin and Mr. Burke Blanchfield of the Bureau of Microbial Hazards, Health Protection Branch, Health Canada for their hard work and assistance with the *Clostridium botulinum* challenge studies.

I would also like to thank Mr. Steven Thibeault from Via-Mer, St-Hyacinthe for supplying the trout trimming mince for this project.

I am grateful to my family for their continued love and support throughout all my studies. I would also like to thank all my friends, particularly Mr. Wai Pong Lai, Ms. Lai Chin Wong, Ms. Siaw Yun Chin, Mr. Kwong Hoe Ng, Mr. Yin Choy Lim, Mr. Soon Hui Tan and Mr. Yee Wei Law who helped me over the years to discover my strength and encouraged my to become who I am today.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1:
INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Canada harvests about 1.6 million tons of fish annually. At dockside, the landed value of the harvest is ~$1.5 billion annually, excluding aquaculture production. The fish harvesting and processing sectors in Canada employ more than 140,000 people of which 55% work in fish processing plants (Blewett and MacDonald, 1998). The seafood processing industry is therefore an extremely important component of the Canadian food industry.

The past decade has been a time of change in the fishing industry. During this period, governments of many nations extended their jurisdiction over fishing territories. At the same time, it became apparent that the resources of the ocean were limited. It is estimated, that currently, about 60% of the available resources are harvested (Regenstein, 1986). There are several major problems facing the growth of the seafood processing industry, including environmental concerns about dumping of fish wastes and dwindling of fish stocks. Thus, if food production from the sea is to be increased, the resources already harvested should be used better, including underutilized fish. It is necessary to investigate the use of underutilized fish species, which are both cheaper and in greater abundance, as well as the use of fish and shellfish processing wastes. Fish and shellfish flesh is often used inefficiently with as much as 50% being discarded as waste each year. These waste products could be incorporated into battered and breaded products to create value-added seafood products resulting in increased profits (Roessink, 1989).

Another problem facing the seafood processing industry is the method of storage and distribution of products. Fish and shellfish flesh are subject to rapid deterioration in quality. To date, freezing is the main method of long term storage (>6 months) to ensure keeping quality and to extend shelf-life. However, with increasing consumer demands for "fresh"

products, and increasing energy costs associated with freezing and frozen storage, the fish processing industry is seeking alternative methods for enhancing the shelf-life, quality, safety and marketability of these products while at the same time reducing the energy costs.

1.2. Fish Products

1.2.1. Mince

Minced fish flesh, commonly referred to as mince, is produced by mechanically deboning deheaded, gutted fish, or a portion thereof. In the mechanical deboning process, the fish is either pressed against a perforated drum by a moving belt or a perforated cylindrical head by an auger. In both systems, the pressure and perforation size are such that the fish flesh, fat and any blood present are forced through the perforations for collection, but the skin, bones and connective tissue do not go through (Lanier and Thomas, 1978).

The starting material for mince production can be fillets removed from fish, the frame remaining after filleting, or deheaded, gutted fish. Mince made from fillets (fillet mince) is lighter in color than that made from deheaded, gutted fish or frames. The desirability of this lighter color varies with the intended use of the mince. A light-colored mince would be more desirable in fish sticks.

Production of fillet mince is often not economically feasible. Another drawback of fillet mince is that meat remaining on the frame after filleting may not be utilized for human consumption, instead, it is used as bait or animal feed, or it is discarded. Discarding frames and the high-quality protein still attached to the frames can be viewed as an ethically questionable practice in light of continuing population pressures on the world food supply. Making mince from frames addresses this concern. Frame mince is dark in color because blood from the backbone and kidney is present (Ingham, 1991). Frame mince is thus suitable for use as a red meat substitute. However, processors have often had little economic incentive to recover the frame meat. It is conceivable that increasing demand for mince, a decreasing demand of frames as bait or feed, increasing disposal costs, or other economic factors could make frame mince production an important profit-making or cost reduction technique.

A third starting material for mince production is deheaded, gutted fish. When fillets of a particular species are not valuable commercially, the use of the entire fish to make mince can be cost effective. This is especially true in cases where mince is used to make surimi. Mince has potential application as an ingredient in many different foods (Martin, 1976). At present, however, it is most widely used to prevent voids and enhance cohesiveness in frozen breaded fish sticks and portions. Mince is also used commercially in fish cakes and seafood dishes as an extender. In addition, mince can be used as a substitute for meat in many traditional foods. However, at present, foods containing mince as a substitute for red meat are not commercially available.

1.2.2. Surimi

Surimi is an intermediate food material that has been used in Japan for centuries to make several foods (Lee, 1984). Its Japanese name, which means "minced fish," refers to a process that was developed in Japan almost 1,000 years ago. Surimi is made by mincing the fish flesh, thoroughly washing it, and then refining and dewatering. Traditionally, surimi was mixed with ingredients such as salt and spices, kneaded, and then steamed, fried or broiled to make kamaboko, tempura and chikuwa respectively (Sonu, 1986). The washing of minced fish flesh removes substantial amounts of water-soluble proteins, vitamins and minerals as well as pigments and odoriferous compounds. The major components of surimi are thus the myofibrillar proteins: actin and myosin. These proteins can readily form gels and can be manipulated by food processors to make foods that have a variety of textures and shapes. The excellent functionality of surimi has led to its use in making seafood analog products such as imitation crab, scallop, shrimp and lobster (Pigott, 1986). Before 1960, surimi was not frozen. It was quickly formulated into heated foods since denaturation occurred during frozen storage. This freeze-denaturation greatly reduced the surimi gel functionality. In 1960, Japanese researchers discovered that by adding antidenaturants (cryopectants) such as sucrose, the freeze-denaturation problem could be practically

eliminated (Sonu, 1986). This discovery changes the surimi-based food industry from one that was batch oriented and dependent on an uneven supply of fresh unfrozen surimi, to one that has a more consistent supply of high quality frozen surimi. The steady supply of surimi allows expansion of the surimi-based foods industry and makes the price of surimi-based foods more consistent (Miyake *et al.*, 1985).

Surimi can be made from a wide variety of fish species. Fresh surimi can be made from species such as tuna, mackerel, croaker and shark. Generally, any fish with good gel forming ability and white meat color can be used to make frozen surimi. The manufacture of surimi alters its composition from the original mince. The main difference between surimi and ordinary minced fish is that it is repeatedly washed with water. Surimi thus contains proportionally less water-soluble protein than mince. Washing of surimi increases the concentration of myofibrillar proteins, which improves gel strength and elasticity. Cryopectants, such as sugar, sorbitol and polyphosphate are added to protect the fish proteins from the denaturing effect of cold temperatures to extend its frozen shelf-life.

When surimi is converted into several surimi-based foods, its composition is again changed. Salt, sugar, starches, egg white, monosodium glutamate and other flavorings are typical ingredients added to surimi in making traditional Japanese foods and modern seafood analogs. Since surimi is essentially bland in flavor due to the washing it receives during production, it can be formulated to have a number of different flavors. including crab, lobster or shrimp. These shellfish analogs have been particularly successful due to the fact that their texture is also very similar. There are also a large number of products which can be produced from frozen surimi, including a number of breaded and battered products, such as fish cakes and fish sticks.

The surimi industry is rapidly expanding due to the fact that surimi has a long shelf-life and is a highly functional protein ingredient of good nutritional quality (Lee, 1986). It is also easily being mass produced, but the largest overall benefit of surimi comes from the

fact that it uses underutilized fish species which are abundant in supply. This is extremely important since the over-exploitation and depletion of the world's most valuable fish stocks still exist, despite attempts to control overfishing (Venugopal and Shahidi, 1994).

1.2.3. Value-Added Seafood

Another important aspect of the minced fish industry is to incorporate 'value-addition' into the process. Value-addition is essentially the process of adding more value to a product from the time that it enters the processing plant to the time it leaves. Lambert (1990) explained that the term 'value-addition' describes the process which changes a product worth $10 in its basic state into a form which makes it worth $15. This is done mainly by using low value or otherwise wasted materials into the formulation to impart different flavors. These flavors are usually associated with more expensive fish and seafood products. Value-added seafood includes battered and breaded seafood, smoked seafood, dried fish and precooked seafood entrees. Battered and breaded seafood includes fish sticks, fish fingers, fish nuggets, fish burgers, shrimp, scallops, and specialty products such as fish and chips, steaks, stuffed fillets and crab sticks. This process can add up to 50% by weight to the end products due to the absorption of oil during the frying process (Roessink, 1989).

Systems for the preparation of battered and breaded seafood include single line and tandem line operations. Single line operations are used when the desired product weight gain attributable to batter and breading is <30%. The seafood is pre-dusted with flour, gluten or dry batter mix and then is soaked in batter, coated with breading, and in some instances precooked (Ingham, 1991). Batters used may based on flour, starch or gums. Batters may also be categorized according to whether they contain leavening agents. Breadings consist of various mixtures of flour, starch and seasonings. The precooking step, typically frying, is done to set the batter and breading. Regardless of whether precooking is done, the food is frozen quickly and held frozen until it is fully cooked immediately prior to consumption.

Tandem line operations follow a similar sequence as the single line except batter and breading are applied twice before the optional precooking step. The pick-up (weight gain caused by batter and breading) in a tandem line exceeds 30%.

Battered and breaded seafood is very important in the fast food business and in institutional food service. These products are also very popular in industrialized countries and are becoming increasingly popular in many Southeast Asian countries as value-added products are synonymous with convenience. Apart from convenience, other reasons for the continued growth of value-added seafood markets are due to: (i) increased ownership of domestic freezers; (ii) two income families with less time to prepare meals; (iii) better educated consumers who require a wider range of available food; (iv) changing consumer tastes; and (v) increased ownership of microwave ovens.

Precooked seafood entrees include a wide range of items. The role of the particular seafood can range from minor, e.g., frozen linguini with clam sauce, to major, e.g., frozen oven-fried fillet dinner. In addition, specialty seafood items, such as gumbo and jambalaya, may be found in grocery freezer cases. The importance of convenient, precooked, frozen meals in the average American's diet is expected to increase in the future.

1.3. Fish Spoilage and Shelf-Life

Fish quality deteriorates rapidly if it is poorly handled and not stored properly. Once fish die, microorganisms present in the gills, gut and skin, in conjunction with the activities of the endogenous enzymes, begin to metabolize compounds, such as free amino acids, sugars, ammonia, trimethylamineoxide (TMAO), creatine, taurine, the betaines, uric acid, anserine, carnosine and histamine, resulting in off-flavors, texture deterioration and discoloration (Ashie *et al.*, 1996). Storage life of freshly cut fish depends on many variables: differences in tissue composition of species; influence of seasons of the year on composition; differences between freshwater and saltwater fish, and the effects of salt on the normal microflora of these fish, as well as varying procurement and holding practices on board fishing vessels.

Spoilage of fish can be subdivided into three categories: (1) enzymatic spoilage, (2) chemical spoilage, and (3) microbiological spoilage. The majority of fish products sold in the supermarket are frozen to ensure a long shelf-life and to prevent all three types of spoilage.

1.3.1. Enzymatic Spoilage

Loss of freshness of fish, which usually precedes microbial spoilage, is primarily attributed to enzymes present in the muscle tissue. When fish is alive, adenosine triphosphate (ATP) is generated under aerobic conditions for muscle contraction. When fish die, anaerobic conditions set in and glycolysis occurs with little ATP being generated. In the absence of ATP, which is the main source of energy for metabolic activity, all biosynthetic activities come to a halt resulting in the inability of cells to maintain their integrity (Ashie *et al.*, 1996). ATP reserves will continue to be used up after the fish is harvested until the reserves are depleted. Alkaline pyrophosphate, instead of ATP, as well as lactic acid are

being produced. In addition, it is believed that nucleotide catabolism is linked to loss of freshness in fish (Figure 1.1). The first five steps of this reaction occur quite rapidly due to the presence of endogenous fish enzymes. The oxidation of hypoxanthine to xanthine and ultimately to uric acid is much slower and is the result of microbial enzyme activity. The depletion of ATP is also associated with rigor mortis, which results from the formation of permanent actomyosin cross-links. This causes stiffening and inextensibility of the fish muscle, which persists until endogenous enzymes "release" the cross-links.

Flavor deterioration is a key component to the breakdown of ATP. Inosine monophosphate (IMP) contributes to the fresh and pleasant odor associated with fish, however, its breakdown will result in loss of this fresh flavor. The breakdown of lipids present in fish is also a cause of off-flavors. Mincing incorporates air into the muscle tissue and promotes lipid oxidation. Enzymes such as lipoxygenase, peroxidase and microbial enzymes initiate lipid peroxidation to produce hydroperoxides (Ashie *et al.*, 1996). These hydroperoxides can undergo further degradation to form aldehydes, ketones and alcohols that result in the development of off-flavors in fish.

Fish, including those species used for surimi, contains TMAO. TMAO is degraded to trimethylamine (TMA) by TMAO reductase in two ways: (1) by autolytic processes and (2) by bacteria present on the fish. TMA is an odorous compound associated with spoiled fish that has been much studied as an indicator of fish spoilage. It reacts with the lipids present in the fish muscle to produce the characteristic "fishy" off-odor of spoiled fish. TMAO may also be decomposed to dimethylamine (DMA) and formaldehyde by TMAO dimethylase. Formaldehyde is believed to cause a sponginess of the fish flesh through cross-linking of muscle proteins during frozen storage.

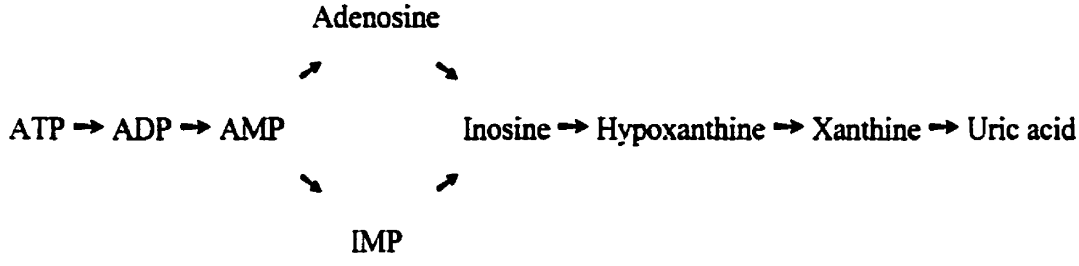

Figure 1.1: Reactions involved in nucleotide catabolism in fish (Flick and Lovell, 1972).

1.3.2. Chemical Spoilage

There are a number of factors associated with the chemical spoilage of fish. These include moisture loss, oxidation, rancidity, loss of volatile flavors, loss of vitamins, and changes in odor and flavor. Fatty fish are more prone to lipid degradation than lean fish. Rancidity is also more pronounced directly under the skin where most fat is located (Bramstedt and Auerbach, 1961). Other than the effect of autolytic enzymes, hydroperoxides are also formed by nonenzymatic methods, namely oxidative rancidity and nonenzymatic browning.

Oxidative rancidity is a major cause of seafood spoilage. The fats of fish are highly unsaturated and become easily oxidized, resulting in rancid off-odors and off-flavors. The mechanism of oxidation of the unsaturated fatty acids is via the formation of free radicals. There are three steps involved in the process: initiation, propagation and termination. Once the reaction has been initiated, free radicals are formed from unstable hydroperoxides, which increase the rate of autooxidation. It has been shown that the oxidation reaction can be catalyzed by various biochemical compounds, including amino acids, heme compounds, organic acids and pigments. Trace metal ions also play a key role in catalyzing these reactions, particularly Cu^{2+}, Fe^{2+} and Fe^{3+}.

Nonenzymatic browning also plays a key role in chemical spoilage of fish. It results in discoloration of the fish via two methods: (1) Maillard reaction, which occurs between sugars and amino acids; and (2) browning reaction, interaction between autooxidative lipid reaction products and proteins (El-Zeany *et al.*, 1975). Khayat and Schwall (1983) suggested that nonenzymatic browning occurs in three steps: (1) formation of lipid peroxides; (2) formation of colorless or slightly colored precursors of brown pigments by interaction of peroxides with active groups of protein, or by interaction of carbonylic peroxide decomposition products with active groups of proteins; and (3) transformation of the colorless or light-colored precursors into brown pigments.

1.3.3. Microbiological Spoilage

Although the flesh of live healthy fish is bacteriologically sterile at the time of harvest, it soon becomes contaminated with bacteria during processing and storage. Sources of contamination include sea water, fish slime, processing equipment, handler, viscera, gill, and skin. Each step in the processing of fish can have an impact on its final microflora. In addition, the habitat of the fish before they are harvested can have profound quantitative and qualitative effects on the microflora of the raw materials. The predominant genera of bacteria on fish at the time of catch are generally Gram-negative cocci such as *Moraxella* and *Acinetobacter*, and Gram-negative rods such as *Pseudomonas*, *Flavobacterium* and *Vibrio* (Nickelson *et al.*, 1980; Liston, 1980). On occasion, Gram-positive cocci and the genus *Micrococcus* will predominate (Gillespie and Macrae, 1975).

During storage of fish in ambient atmosphere on ice or at refrigeration temperature, the genera *Pseudomonas*, *Moraxella* and *Achromobacter* become dominant (Gillespie and Macrae, 1975; Lerke *et al.*, 1965; Shewan *et al.*, 1960). Some *Pseudomonas* and *Achromobacter* species will produce large amounts of TMA (Banwart, 1989; Laycock and Regier, 1971). When fish are harvested, these bacteria rapidly attack all tissue constituents. Furthermore, since these bacteria live on the cold-blooded fish at rather low ocean temperatures, they are well adapted to cold and continue to grow even under common refrigeration conditions. Generally, spoilage occurs slowly if the surface area is low and more rapidly when the surface area is high. Therefore, minced fish will spoil more rapidly than whole fish or fish fillets. The mincing process could also lead to an increase in microbial contamination through lack of temperature control and cross-contamination.

Microorganisms contribute to the degradation of fresh fish in a number of ways. They provide bacterial enzymes necessary for certain degradative processes, such as the formation of TMA from TMAO by providing the bacterial enzyme trimethylamine oxidase. Other compounds produced as a result of microbial activity include hydrogen sulfide,

dimethylsulfide and methyl mercaptan from sulphur containing amino acids; various amines and ammonia from amino acids; carbonyl compounds from lipids; and indole, skatole, putrescine and cadaverine from proteins (Avery and Lamprecht, 1988; Herbert and Shewan, 1976; Smith *et al.*, 1984; Watts and Brown, 1982).

In the manufacture of mince, surimi and value-added seafood, a supply of high quality raw material is absolutely essential for making products of high quality.

1.4. Modified Atmosphere Packaging

In recent years, increasing concerns about health and the environment have resulted on greater attention to how food is produced, processed, packaged, stored, distributed and consumed. The current growing demand for "near-fresh" quality and shelf-stable products has spurred the developments of many innovative processing and preservation techniques. Foremost among these are retortable pouches, aseptic packaging, controlled-atmosphere storage and modified atmosphere packaging. The last being one of the most promising and most extensively studied at the present time.

Young *et al.* (1988) defined modified atmosphere packaging (MAP) as "the enclosure of foods in high gas barrier materials in which the gaseous environment has been changed or modified to slow respiration rates, reduce microbial growth, and to retard enzymatic spoilage, with the intent of extending shelf-life." A modified atmosphere, as the name implies, is one in which the normal composition of air is changed or "modified" within a package. Since the shelf-life of meat and other food products is affected by the presence of oxygen (O_2) and growth of aerobic spoilage microorganisms, atmosphere modification usually results in a reduction of the oxygen content in the package, and an increase in nitrogen (N_2) and carbon dioxide (CO_2).

The use of modified atmospheres for shelf-life extension of food is not new. In the 19th century, scientists discovered that increasing CO_2 levels and reducing O_2 levels retarded catabolic reactions in respiring foods, and slowed the growth of aerobic spoilage microorganisms (Smith *et al.*, 1990a). European markets, have in fact, effectively commercialized MAP. It is a rapidly expanding technology due to the fact that energy costs associated with other methods of preservation, such as freezing and drying, are relatively high. There is also an increasing demand for additive- and preservative- free products.

1.4.1. MAP Technology

MAP relates to the modifications of O_2 and CO_2 levels compared to the normal composition of air (20.9% O_2, 79% N_2 and 0.1% CO_2) before storage. Their concentrations are allowed to fluctuate as a function of time, temperature and package transmission rates. The effectiveness of MAP is limited by the manufacturing practices that are employed. It does not overcome hygienic abuses in the manufacture and handling of food products, however when it is applied correctly, it can slow the natural deterioration of perishable foods (Campden Technical Manual, 1992). Therefore, it is necessary to start with high quality products in order for MAP technology to be truly effective. Three methods commonly used by the food industry to modify the gas atmosphere within a packaged product are vacuum packaging, gas packaging and oxygen absorbent technology.

1.4.1.1. Vacuum packaging

Vacuum packaging is one of the most common methods of food preservation and it is used extensively by the meat industry. It involves packaging the product in a film of low oxygen permeability, the removal of air from the package, and the application of a hermetic seal (Smith *et al.*, 1990a). The headspace O_2 is usually reduced to less than 1% under good vacuum conditions, while 10-20% CO_2 is produced from tissue and microbial respiration. These conditions of low O_2 and elevated CO_2 help extend the shelf-life of meat and other food products by inhibiting the growth of normal aerobic spoilage microorganisms, particularly *Pseudomonas* and *Alteromonas* species (Gill and Tan, 1980).

1.4.1.2. Gas packaging

Gas packaging is a technique in which various combinations and mixtures of CO_2, N_2 and O_2 are used, depending on the product. The process involves removing the air out of the package, similar to vacuum packaging, then back flushing with the desired mixture of gases.

The levels and mixtures of gases (O_2, CO_2 and N_2) used depend primarily on the food product. For example, in cured meat products, O_2 is not necessary and can even be detrimental to product color. Therefore, it is necessary to package cured meats in an O_2 free mixture of CO_2 and N_2, or in 100% of either gases. In fish, autoxidative changes lead to the formation of low molecular weight aldehydes, ketones, alcohols and carboxylic acids. Therefore, the gas mixture depends on the fat content of the fish. Low fat fish can be packaged in 60% CO_2 and 40% O_2 while high fat fish need to be packaged in an O_2 free environment to prevent rancidity problems.

Carbon dioxide (CO_2) is the most commonly used and most effective gas used in MAP. It is bacteriostatic and fungistatic. It retards growth of spoilage microorganisms and reduces oxidative rancidity of fat by displacing O_2. It also prevents insect growth and it is highly soluble in fat and water where it forms carbonic acid (Brody, 1989). There are several factors that influence the antimicrobial effect of CO_2, specifically microbial load, gas concentration, temperature and packaging film permeability (Smith *et al.*, 1990b).

The protective mechanism of the CO_2 atmosphere against microbial growth is not well understood. However, there are several theories for the mechanism of microbial inhibition by CO_2. Four theories of the mechanism are attributed to the preservative effects of CO_2 in foods. Most likely, the preservative effects are due to a combination of the following factors:

1. Displacement of O_2 by CO_2 inhibits the growth of aerobic microorganisms and results in a delayed growth phase (prolonged lag phase) while there is a shift of dominant microflora that tend to be antagonistic to spoilage bacteria (Callow, 1932; Price and Lee, 1970).
2. Hydration of CO_2 to carbonic acid may result in acidification of the tissue (Aichin and Thomas, 1975; Becker, 1933; King and Nagel, 1967). It should be noted, however, that the hydration of CO_2 to carbonic acid is estimated to be only 1-2 % (Knoche, 1980; Brinkman *et al.*, 1933; Quinn and Jones, 1936). Carbonic acid dissociates rapidly, and forms bicarbonate (HCO_3) and hydrogen ions (H^+). Yet, lowering the extracellular pH to the same extend by using HCl did not give such high bacterial and mold inhibition.
3. Either CO_2 or its ions may alter the bacterial cell permeability characteristics, specifically by increasing fluidity of membrane fatty acids and/or the carbamination of proteins (Krough, 1919; Sears and Eisenberg, 1961; Castelli *et al.*, 1969; Fox, 1981). This is a possible mechanism to inhibit aerobic spore germination (Sears and Eisenberg, 1961; Enfors and Molin, 1978).
4. Metabolic pathways may be affected by the presence of CO_2 and result in altered bacterial enzymatic activity, e.g., an increased rate of succinate formation by *Bacillus*, inhibition of isocitrate dehydrogenase and malate dehydrogenase by *Pseudomonas*, and interference with hydrolases and thus autolysis (Elsden, 1938; King and Nagel, 1975; Mitsuda *et al.*, 1980; Smikahl, 1985). This may be the result of either altered solubility of proteins and/or feedback inhibition mediated by enhanced CO_2 solubility at reduced temperatures (Delente *et al.*, 1969; Krebs and Roughton, 1948).

CO_2 is highly soluble in water and will form carbonic acid. It will consequently reduce the pH of the fish, which is normally at 6.3, down to a range of 5.7-5.8 (Brody, 1989). This dissolution phenomenon is dependent on temperature, moisture content and concentration of CO_2. As temperature increases, solubility decreases and microbial growth

increases (Ogrydziak and Brown, 1982). CO_2 has been proven to be more effective against Gram-negative bacteria than Gram-positive bacteria (Yasuda *et al.*, 1992).

The overall effect of CO_2 with appropriate temperature control increases both lag phase and generation time of spoilage microorganisms (Smith *et al.*, 1990a). The predominant Gram-negative psychrophiles in cold water fish are *Moraxella*, *Acinetobacter*, *Pseudomonas*, *Flavobacterium* and *Vibrio* (Ward, 1989). CO_2 is most effective against aerobic spoilage microorganisms but it has little or no effect on facultative organisms such as *Enterobacteriaceae*, *Brocothrix thermosphacta* or microaerophilic lactic acid bacteria (Brody, 1989). Anaerobic bacteria, as well as pathogens, such as *Salmonella* spp., *Staphylococcus aureus*, *Campylobacter* spp., *Yersinia enterocolitica* and *Clostridium* spp. are not markedly affected by CO_2 and MAP anaerobic conditions may even favor their growth (Smith *et al.*, 1990a).

The numbers, types and age of the microbial population greatly affect the antimicrobial properties of CO_2 (Brody, 1989). As bacteria move from the lag phase to the log phase of growth, the inhibitory effects of CO_2 are reduced. Therefore, the earlier the packaging, the greater the effectiveness of CO_2. MAP does not improve the quality of a product but maintains it and helps retard its further degradation. A concentration of 20-60% CO_2 is required in the package headspace to be effective against aerobic spoilage microorganisms. It has been shown that the inhibitory effect of CO_2 increases linearly with increasing concentration, but little effect above 60% (Gill and Tan, 1980; Brody, 1989). Storage temperature also affects bacterial inhibition by CO_2. The antimicrobial activity of CO_2 is enhanced at lower storage temperature, which is due to the increased dissolution of CO_2 in the aqueous phase of the food product.

Nitrogen (N_2) is used in gas packaging as an inert filler gas and to prevent package collapse, especially when CO_2 dissolves into the food product (Farber, 1991). It has no effect on food and has no antimicrobial properties. It can also be used to replace O_2 in foods to

prevent oxidative rancidity, especially in foods with low water activity (a_w), e.g., peanuts.

Oxygen (O_2) is generally not used in gas packaging applications, except in meat products where it is used to maintain the red color, or "bloom" associated with fresh red meats. O_2 can also be used in low concentration in the packaging of respiring products.

1.4.1.3. Oxygen absorbent technology

While gas packaging has been used to slow down or inhibit aerobic deteriorative changes in food products, aerobic spoilage can still occur in such products depending on the level of residual O_2 in the package headspace. The level of residual O_2 in gas packaged products could be affected by a number of factors: (a) O_2 permeability of the packaging material. (b) ability of the food to trap air, (c) leakage of air through poor sealing, and (d) inadequate evacuation and/or gas flushing (Smith, 1994). A novel and innovative method of O_2 control and of atmosphere modification involves the use of oxygen absorbents.

Oxygen absorbents can be defined as "a range of chemical compounds introduced into the MAP package (not the product) to alter the atmosphere within the package" (Smith. 1994). Developed in Japan, oxygen absorbents were first marketed by the Mitsubishi Gas Chemical Company, under the trade name "Ageless". Ageless absorbent consists of a range of gas scavenger products designed to reduce O_2 level to less than 100 p.p.m. in the package headspace (Smith, 1994). The sachet material is highly permeable to oxygen and water vapor. Under appropriate humidity condition, the basic system which is made up of finely divided powdered iron uses up residual O_2 to form nontoxic iron oxide (rust). The mechanism of oxidation is as follows:

$$Fe \rightarrow Fe^{2+} + 2e^-$$

$$\frac{1}{2} O_2 + H_2O + 2e^- \rightarrow 2OH^-$$

$$Fe^{2+} + 2OH^- \rightarrow Fe(OH)_2$$

$$Fe(OH)_2 + \frac{1}{2} O_2 + \frac{1}{2} H_2O \rightarrow Fe(OH)_3$$

Oxygen absorbent technology was developed by the Japanese, and has become a major method for food preservation. It has been used widely to extend the mold-free shelf-life of breads, pizza crusts and cakes, as well as to prevent oxidation of fats in potato chips, dried fish, beef jerky, semi-moist cookies and chocolates (Smith, 1993).

1.4.2. Use of MAP to Preserve Seafood Products

MAP is an effective method to extend the shelf-life of fresh fish and seafood. When packaged in air, the shelf-life of seafood is limited by the growth of Gram-negative, psychrotrophic strains of *Pseudomonas*, *Achromobacter*, *Flavobacterium* and *Moraxella* spp. (Ashie *et al.*, 1996). Even at 0°C, eviscerated fish and fish fillets usually spoil within 7-10 days of storage (Eklund, 1993). Growth of these microorganisms is inhibited when packaged under modified atmospheres.

Packaging under modified atmospheres is also effective at delaying oxidative rancidity. In white fish, crustaceans and shellfish products, gas mixtures containing 35-45% CO_2, 25-35% O_2 and 25-35% N_2 are recommended (Campden Technical Manual, 1992). Mixtures containing 35-45% CO_2 and 55-65% N_2 are recommended for oily fish products since they are more susceptible to oxidative rancidity.

In order for MAP fish products to be effective, high quality fish and seafood products should be used. The shelf-life of these products depends upon the species, fat content, initial microbial load, gas mixture and storage temperature. It is important to maintain good

hygiene and proper temperature storage condition in order for MAP to be effective.

Although the U.S. Food and Drug Administration (FDA) does not currently endorse modified atmosphere storage of seafood, Cann (1984) recommended that for white fish, scampi, shrimp and scallops, a 40% CO_2/30% O_2/30% N_2 environment provides the best results. For the fatty fish, e.g., salmon, trout, herring, mackerel and smoked fish products, a 60% CO_2/40% N_2 atmosphere is the most desirable gas atmosphere.

1.4.3. Microbiological Safety of MAP

The safety of MAP is of major concern since the reduced O_2 will inhibit bacterial growth but may permit the growth of other pathogenic microorganisms. The growth of the normal spoilage microorganisms (indicators of spoilage) will be delayed and thus there is a shift in characteristic spoilage microflora. Odors normally associated with spoilage will not develop and may create a false confidence in product safety. MAP products may also be exposed to temperature abuse during storage and distribution, which may increase the risk associated with MAP foods.

Temperature control is probably the most critical consideration in MAP. The safety of MAP products, which are susceptible to contamination with *Clostridium botulinum* spores, is of public health concern, particularly under temperature abuse conditions. The ability for *C. botulinum* to grow under anaerobic conditions, as well as the stimulative effect of CO_2 on spore germination, have justified these concerns. It is known that *C. botulinum* can grow at temperature as low as 3.0°C (Graham *et al.*, 1997). Abusive handling for even a few hours can result in the outgrowth of *C. botulinum* spores and for this reason, strict temperature control must be maintained.

Many believe that O_2 should be included in the package headspace of high risk foods to minimize this potential hazard. However, the presence of O_2 in the package should not be the only safety measure. Furthermore, some studies have shown that the inclusion of O_2 in the package headspace may have little or no effect on the growth of *C. botulinum* in raw fishery products (Eklund, 1982). More research into the biochemical indicators is needed to develop criteria for acceptability of MAP seafood. In addition, such criteria may differ from one species to another since aerobic shelf-life and spoilage patterns can vary widely.

1.5. Safety of Fish Products

Seafood is a commodity that has a good history of food safety but it has the potential to cause a wide spectrum of public health problems due to contamination and growth of pathogenic bacteria during production and distribution from the point of harvest to final preparation. An understanding of the major foodborne pathogens and associated problems has enabled control measures to be implemented that effectively eliminate or greatly reduce the risk of human illness from these pathogens. Food sanitation and quality control measures that ensure the safety of seafood from harvest to consumption must continue to be developed in the future to ensure a continued high level of safety. Seafood-borne disease organisms can be divided into several groups based on the source of contamination.

Human foodborne illness is often caused by bacterial pathogens from fecal contamination of food either directly by ill or by asymptomatic carriers such as food handlers or by infected domestic animals raised for food. Man, as a carrier of common bacteria such as *Salmonella* spp. and *Shigella* spp., can contribute to human illness by direct fecal-oral transmission of these types of pathogens. Seafood can act as a fomite. Even if the level of contamination is not great, an infectious dose can often be quite low if no final heat process is applied after preparation prior to consumption. This first category of risk includes eating raw fish (sashimi) as well as other aquatic food animals such as molluscan shellfish.

Exposure of seafood species to sewage-contaminated water may result in contamination of the seafood with pathogenic bacteria and viruses of fecal origin. Flatfishes caught near effluent discharge points in the Baltic Sea were found to contain *Salmonella*, a genus that causes a high percentage of foodborne illnesses (Wuthe and Findel, 1972). *Clostridium perfringens*, a spore-forming anaerobe capable of causing gastroenteritis, was found in all gut samples from fish caught near sewage outfalls in Puget Sound, Washington (Matches *et al.*, 1974). Seafood harvested from polluted water, or raised in aquaculture systems could be considered as vectors rather than fomites because of the close contact of

the living product with man and his agricultural livestock in closed or confined harvest areas. This second group of contaminants also includes those found in the natural pristine marine or fresh water environment. These include *Vibro* spp. and *C. botulinum* which exist in the water column or sediments of the harvest area.

However, the greatest source of adulteration of seafood with enteric pathogens is due to unsanitary practices during product handling and processing. Products that are fully cooked or otherwise processed are also subject to subsequent cross-contamination. An increase in the numbers of these organisms by time-temperature abuse often coincides with unsanitary handling, thus increasing the potential for disease. *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, pathogenic forms of *Escherichia coli* and *Y. enterocolitica* are commonly found in the marine environment and the chance of human infection from seafood is increased with product abuse (Ingham, 1991).

1.5.1. Safety of Value-Added Fish Products

The microflora of battered and breaded seafood can arbitrarily be divided into organisms naturally associated with the seafood, organisms associated with the batter and breading ingredients, and organisms associated with processing workers and equipment. The microbiology of batter and breading mixtures has been thoroughly reviewed by Fung (1983). The major ingredients in batter and breading mixtures are flour, starch, spices, milk, eggs and water. Spices can be heavily contaminated with bacterial cells, spores of bacteria and molds. Even though some spices contain compounds with antimicrobial activity, the addition of spices to a cooked food can contaminate that food and possibly lead to foodborne illness. Spores of the foodborne pathogens *C. perfringens* and *Bacillus cereus* have been frequently found on spices (Powers *et al.*, 1976). The heating of foods containing spices will decrease the microbial load of the spices.

The application of sound food handling and processing techniques is necessary to ensure the microbiological safety, and keeping quality of battered and breaded seafood. These techniques are also vitally important for precooked seafood entrees. Good manufacturing practices including sanitary handling, thorough heat processing, proper use of high-quality ingredients, and avoidance of post-cooking cross-contamination must be adhered in preparing these entrees. The current trend in favor of precooked refrigerated (non-frozen) foods has placed an added emphasis on these practices in the production of safe value-added seafood products.

Ready-to-eat seafood is usually subjected to post-processing contamination. Pathogenic bacteria which may be introduced into these seafood products include *Aeromonas hydrophila*, *Vibro parahaemolyticus*, *S. aureus*, *L. monocytogenes* and *C. botulinum* (Ingham and Potter, 1988).

A. hydrophila has been isolated from oysters, poultry and a variety of red meats (Abeyta *et al.*, 1986; Okrend *et al.*, 1987). It can grow on foods at cold temperatures as low as 3°C (Eddy and Ketchell, 1959). Since the early 1970s, *A. hydrophila* has been cited as a potential cause of foodborne gastroenteritis. Because *A. hydrophila* does not grow well in levels of NaCl above 1.5% (Ingham and Potter, 1988; Palumbo *et al.*, 1985), it is most likely to be a contaminant of fish from fresh or estuarine waters.

Other pathogenic bacteria naturally present in the pre-harvest environments of seafood include *V. parahaemolyticus* and *Vibro vulnificus*. These species are found in warm waters and require NaCl at levels of 2-3% for optimal growth. Foodborne illness caused by *V. parahaemolyticus* and *V. vulnificus* is most likely to be associated with ingesting raw seafood, or as a result of cross-contamination of cooked seafood. *V. parahaemolyticus* is halophilic, therefore it will grow well in high-salt surimi. However, it does not grow well at refrigeration temperatures (Ingham and Potter, 1988).

Seafood, regardless of species, can support the growth of *S. aureus* when contaminated and because they are high in protein content and thus fulfill the nutritional needs of the microorganisms. *S. aureus* is a toxin producing, Gram-positive coccus which occurs due to cross-contamination or mishandling. It has also been shown to grow well in high salt conditions but it does not grow well at refrigeration temperatures. Typical outbreaks of staphylococcal seafood poisoning occur in cooked products, such as smoked fish or crab, since heating destroys competitive microorganisms and allowing staphylococci to predominate. There are five serological types of staphylococcal enterotoxin designated A-E. Most food outbreaks involve types A or D (Sperber, 1977).

Evidence has also been presented to suggest that the pathogenic bacterium *L. monocytogenes* can frequently contaminate seafood during processing (Weagant *et al.*, 1988). An outbreak was reported in Finland where febrile gastroenteritis in 5 healthy persons was associated with the consumption of vacuum packed cold-smoked rainbow trout containing *L. monocytogenes* (Miettinen *et al.*, 1999). Listeriosis was also a possible cause of a New Zealand outbreak through the consumption of raw fish and shellfish (Lennon *et al.*, 1984).

Fish may also contain spores of *C. botulinum* type E, an anaerobic spore-forming bacterium found in marine and aquatic environments, such as sediments and the intestinal tract of fish. It can grow and produce toxin at temperatures ranging from 3.0°C to 45°C (Banwart, 1989; Graham *et al.*, 1997). Many refrigerators do not maintain temperature <3.0°C, so growth of *C. botulinum* type E is a threat when fish is stored under anaerobic conditions. Outbreaks of botulism caused by *C. botulinum* type E have involved smoked fish, vacuum-packaged fish and canned fish (Ingham, 1991).

1.5.2. Concerns Associated with MAP Seafood Products

The use of MAP technology in the seafood industry is increasing, but there is still concern about the public health safety of value-added seafood products packaged under modified atmospheres. With many modified atmospheres containing moderate to high levels of CO_2, the aerobic spoilage organisms which usually warn consumers of spoilage are inhibited, while the growth of pathogens may be allowed or even stimulated. Major safety concerns that need to be addressed include the potential for growth of psychrotrophic facultative/anaerobic pathogens found in seafood products. Studies have shown that a high level of CO_2 has an inhibitory effect on *V. haemolyticus*, *S. aureus* and *A. hydrophila* at refrigeration temperature (Kimura *et al.*, 1997, 1999; Devlieghere *et al.*, 1998; Doherty *et al.*, 1996). However, growth of *L. monocytogenes* and non-proteolytic *C. botulinum* type E in MAP seafood product still need to be addressed to ensure the microbiological safety of these products.

1.5.3. *Listeria monocytogenes*

L. monocytogenes is a Gram-positive, non spore-forming, facultative rod, motile by peritrichous flagella, and exhibiting tumbling motility under a light microscope in a wet mount at 25°C. It is a pathogenic and psychrotrophic microorganism, capable of growing between -0.4 and 50°C (Farber and Peterkin, 1991). The colonies demonstrate a characteristic blue-green sheen by obliquely transmitted light. *L. monocytogenes* is catalase positive, oxidase negative, and expresses a β-hemolysin which produces zones of clearing on blood agar.

Listeria is relatively heat resistant but usually is not present in foods receiving adequate heat treatment. The presence of *L. monocytogenes* in cooked seafood indicates cross-contamination of the product or underprocessing. *Listeria* is aerobic under most

circumstances but can be facultatively anaerobic and thus grows well under reduced levels of oxygen in MAP packaged products. *Listeria* can also survive and grow under refrigeration temperature. These bacteria also survive freezing well, thus making adequate cooking and prevention of recontamination extremely important.

Listeria is commonly identified by serotyping. Types 1 to 7 are known, with types 1a, 1b and 4b predominating as both environmental and clinical isolates. The serotyping scheme is based on both somatic and flagellar antigens. Phage typing has also been employed as a method of further identifying isolated strains. There are currently 27 phages used in the typing system of Audurier (Mclaughlin *et al.*, 1986).

L. monocytogenes is an interesting pathogen because it is a facultative intracellular parasite. The organism enters the body through the intestine and has a variable incubation period that may be as short as 1 day to a month or longer (Kverberg, 1991). The ingested cells enter the body through ileal villi cells and are subsequently taken up by macrophage cells in the bloodstream. Instead of being digested, the engulfed cells multiply inside the host cell until the macrophage bursts and liberates the *L. monocytogenes* cells to repeat the process. This causes the transitory flu-like symptoms often reported at the initial stage of disease. The enteric phase of the disease is not consistent; some report upset stomach and diarrhea whereas other victims do not display these symptoms. The actual disease, known as listeriosis, does not occur until a severe form of septicemia, encephalitis, lesions or meningitis develops. All of these forms of listeriosis may accompany infection of those who are not immunocompetent (Lovett, 1989). The susceptible groups include pregnant women and their fetuses, cancer patients and others undergoing immunosuppressive therapy, as well as diabetics, alcoholics, cirrhotics, people with cardiovascular, renal collagen, neoplastic diseases, and the elderly (Nieman and Lorber, 1980). Mortality is high among those infected as evidenced by a 29% death rate among patients in a New England outbreak associated with fluid milk (Fleming *et al.*, 1985).

Listeria spp. have had little documented association with seafood until very recently. It has long been known that *Listeria* is widely distributed in the environment. It is ubiquitous in nature and has been isolated from soil, water, humans, and a variety of animals such as cattle, sheep, goats and poultry (Gray and Killinger, 1966). There is little known association between seafood and listeriosis. An outbreak of perinatal listeriosis was reported from three obstetric hospitals in Auckland, New Zealand. Most of the 22 cases were due to strain 1b. The cause of the outbreak was not discovered. Consumption of raw shellfish and raw fish may have played a role (Lennon *et al.*, 1984).

1.5.3.1. Factors influencing growth of *L. monocytogenes*

Some of the factors affecting growth of *L. monocytogenes* include: temperature, pH, a_w, atmosphere and food additives.

L. monocytogenes has a temperature growth range of -0.4 to 50°C (Farber and Peterkin, 1991). Although its optimum growth temperature is between 30-37°C, it is also capable of growing at refrigeration temperatures. In laboratory media, *L. monocytogenes* grows very slow at 1°C, however, at slightly higher temperatures, i.e., 3-6°C, the growth rate increases such that a final population of ~10^8 CFU/ml is attained after several weeks of incubation (El-Shanawy and Marth, 1988).

The minimum pH at which *L. monocytogenes* is capable of growing is still debatable. Parish and Higgins (1989) reported that *L. monocytogenes* was capable of growing in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) at pH values from 4.5 to 7.0 but with no growth at pH 4.0 or less when incubated at 30°C. Farber and Peterkin (1991) suggested that the minimum pH required for the initiation of growth ranges from 5.0 to 5.7 at 4°C and 4.3 to 5.2 at 30°C. However, Ita and Hutkins (1991) indicated that the organism would survive under certain conditions at pH 3.5 for 24 hours. They also concluded that

inhibition of *Listeria* by acid is caused by specific effects of the undissociated acid on cellular processes and not by a decrease in intracellular pH. It is also quite resistant to alkaline pH and has been shown to grow in liquid media at pH 9.6 (Seeliger, 1961).

The ability of five strains of *L. monocytogenes* to initiate growth at five different temperatures in brain-heart infusion (BHI) broth and adjusted to various a_w values with either NaCl, sucrose or glycerol was investigated by Farber *et al.* (1992). Glycerol was found to be the least toxic of the three solutes. *L. monocytogenes* was capable of growth at 30°C at an a_w of 0.90 (reduced by glycerol) compared to 0.93 and 0.92 for sucrose and NaCl adjusted media respectively. It was also reported that the minimum a_w for growth increased as incubation temperature decreased.

The gas atmosphere also affects the growth of *L. monocytogenes*. Since *L. monocytogenes* is a facultative organism, growth may be enhanced under low oxygen concentrations. It has been reported that modified atmosphere does not completely inhibit the growth of *L. monocytogenes* but may extend its lag phase and generation time (ICMFS, 1996).

McClure *et al.* (1991) found that at 30°C, increasing the $NaNO_2$ level to 400 µg/ml had little effect in delaying growth of *L. monocytogenes* at or above pH 6.0. At pH <6.0, $NaNO_2$ had some inhibitory effect, including the lowest concentration of 50 µg/ml. No growth was observed at pH 5.3 in the presence of $NaNO_2$ when incubated at 20°C or lower.

1.5.3.2. *L. monocytogenes* in food

L. monocytogenes has been found in a wide range of food products, including fish and seafood products, milk and dairy products, fresh and processed meat, and fresh produce including coleslaw. However, it is only within the past few years that *L. monocytogenes* has

been established as a foodborne pathogen. It represents a major problem to food processors since it is widespread in the environment and it can grow at refrigeration temperatures. Its occurrence in food is usually due to cross-contamination of ready-to-eat products by food handlers or by surfaces during slicing and packaging (Oh and Marshall, 1994).

L. monocytogenes has been found in a wide range of dairy products, primarily cheese. Levels as high as 10^7 CFU/ml have been documented in cheese (Farber and Peterkin, 1991). In meat, most contamination usually occurs on the surface. However, Johnson *et al.* (1990) found *L. monocytogenes* in the interior muscle core samples of beef, pork and lamb roasts. Chicken could also be heavily contaminated with *Listeria* (Bailey *et al.*, 1990). Although many different types of vegetables have been analyzed for the presence of *L. monocytogenes*, only potatoes and radishes appear to be regularly contaminated (Heisick *et al.*, 1989). Sources of contamination include soil, water, animal manure, decaying vegetation and effluents from sewage treatment plants (Beuchat *et al.*, 1990). Since *L. monocytogenes* is widespread in the environment, fish and seafood products have also been targeted as potential sources of this organism. Although fish products have not been studied extensively for *Listeria* compared to other foods, products found to contain *L. monocytogenes* include shrimp, crab, lobster, fin fish and surimi-based products.

The minimum number of pathogenic *L. monocytogenes* cells which must be ingested to cause illness in both healthy or immunocompromised individuals are not known. The number of cells required to cause illness varies depending on the strain and host susceptibility.

The tolerance levels for *L. monocytogenes* in foods set by Health Canada are as follows: (i) a zero tolerance level in foods that have been linked to listeriosis outbreaks, e.g., coleslaw, cheese, etc.; (ii) a zero tolerance for foods with a shelf-life of more than 10 days that are capable fo supporting its growth, e.g., vacuum packaged meats; and (iii) a tolerance level of less than 100 CFU/g in frozen foods as long as the food was processed and packaged

under Good Manufacturing Practices (GMP).

1.5.3.3. Control of *L. monocytogenes* in food

There are a number of methods to control the growth of *L. monocytogenes* in food. These include thermal processing, temperature control, as well as other novel methods of control such as bacteriocins and modified atmosphere packaging.

The extent to which *L. monocytogenes* is heat stable is debatable. This controversy in the thermostability can be attributed to two main problems. First, a phenomenon called 'heat shock response' was associated with *L. monocytogenes*. It has been shown that if *L. monocytogenes* is exposed to sublethal temperatures around 44-48°C, the cells acquired an enhanced thermotolerance (Farber and Brown, 1990). Also, cells grown at higher temperatures have a higher thermotolerance. The second problem is related to the method of recovery of heat-stressed organisms. It has been shown that significantly more heat-stressed cells can be recovered in an anaerobic environment than in the presence of oxygen (Knabel *et al.*, 1990). The oxygen sensitivity of heat-stressed *L. monocytogenes* is thought to be due to the inactivation of the enzymes catalase and superoxide dismutase during heating. Doyle *et al.* (1987) concluded that *L. monocytogenes* can survive a heat treatment of 72.2°C for 15.4 seconds, which may be used for High-Temperature-Short-Time (HTST) pasteurization of fluid milk.

The ability of *L. monocytogenes* to grow at refrigeration temperatures poses a serious threat to the food industry (Ryser and Marth, 1991). It reached a higher population on raw and cooked catfish and shrimp than on beef or chicken stored aerobically at 4°C (Shineman and Harrison, 1994). It was discovered that some of the differences in growth rates could be attributed to the inherent pH differences of the product tissues. Beef and chicken both have a natural pH of ~5.7, while shrimp is ~pH 7.6. Hangard-Vidaud *et al.* (1989) isolated *L.*

monocytogenes strains from 10% of fresh trout samples, cooked shrimps, crab and smoked salmon. Although the organism was present at low levels, their study showed that it is capable of growing at low temperatures during storage. Freezing has the ability to extend the shelf-life of foods 5 to 50 fold compared to refrigeration (El-Kest *et al.*, 1991). Although freezing is not considered bactericidal, extended frozen storage can result in death of bacterial cells due to cellular damage.

Bacteriocins are biologically active proteins produced by some strains of bacteria including naturally occurring foodborne organisms (Klaenhammer, 1988; Daeshel, 1989). These proteins are lethal against other bacteria, and can be produced by a wide variety of organisms, including *Bacillus*, *Bacteriodes*, *Brucella*, *Carnobacterium*, *Caulobacter*, *Citrobacter*, *Clostridium*, *Corynebacterium*, *Enterobacter*, *Escherichia*, *Halobacteria*, *Klebsiella*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Listeria*, *Micrococcus*, *Mycobacterium*, *Neisseria*, *Pasteurella*, *Pedicoccus*, *Propionibacterium*, *Proteus*, *Pseudomonas*, *Salmonella*, *Sarcina*, *Serratia*, *Staphylococcus*, *Streptococcus* and *Vibrio* spp. (Barefoot *et al.*, 1992). Numerous bacteriocin-producing lactic acid bacteria were found inhibitory to *L. monocytogenes* such as bacteriocins produced by *Carnobacterium piscicola* and *Carnobacterium divergens*, pediocin produced by *Pediococcus acidilacti* and sakacin produced by *Lactobacillus sake* strain Lb 706 (Pilet *et al.*, 1995; Nielsen *et al.*, 1990; Schilinger *et al.*, 1991). In addition to lactic acid bacteria, Larsen and Gundstrup (1965) reported that certain strains of *Bacillus subtilis* and *Bacillus pumilus* could prevent growth of *L. monocytogenes* on tryptose agar at 37°C.

The effects of modified atmosphere packaging on the growth of *L. monocytogenes* have not been studied as extensively. There are some concerns since it is a facultatively anaerobic microorganism, and therefore would be able to grow in low oxygen concentration. However, it was found that an atmosphere of 100% CO_2 has a significant inhibitory effect on the growth of *L. monocytogenes*, which is enhanced at lower temperatures (Razavilar and Genigeorgis, 1992).

A variety of preservatives are employed to control *L. monocytogenes* in food. Some examples of these include potassium sorbate, sodium propionate, sodium benzoate, sodium nitrite, antioxidants and lysozyme. It was also found that these preservatives provided a synergistic effect when used in combination with each other, enhancing the inhibitory properties of the individual compounds.

1.5.4. *Clostridium botulinum*

C. botulinum is a Gram-positive spore former that can grow between pH 4.6 and 8.5. It is a catalase negative, anaerobic spore-forming rod that produces a highly potent neurotoxin. These characteristics allow *C. botulinum* to survive high temperatures and multiply in absence of oxygen. The disease caused by the toxin is called botulism, and it is caused by a variety of organisms in the genus *Clostridium*, primarily *C. botulinum*, but also *Clostridium barati* and *Clostridium butyricum*. *C. botulinum* is classified into seven types: A, B, C, D, E, F and G, based on differential neutralization with type-specific antitoxin reagents. Group I consists of the proteolytic types A, B and F, and group II consists of the non-proteolytic types B, E and F.

Human botulism is almost always of type A, B or E (Hatheway, 1993). Botulinum neurotoxin acts by blocking the release of acetyl choline at the neuromuscular junction in a three-step process: (1) the toxin molecule binds to receptors on the nerve ending; (2) the toxin molecule, or a portion of it, is internalized; and (3) within the nerve cell, the toxin interferes with the release of acetylcholine (Simpson, 1986). There are essentially four forms of botulism: (i) the "classic" form caused by preformed toxin in the food; (ii) wound botulism, which is very rare but occurs like other clostridial wound infections; and (iii) infant botulism, which occurs as a result of ingestion of botulinal spores and subsequent germination, growth and toxigenesis in the intestine. The fourth form has yet to be classified and includes cases of unknown origin, and adult cases that resemble infant botulism

(Hauschild, 1989).

Botulism is a rare form of foodborne disease but its occurrence causes great concern because of its life-threatening nature. The onset of symptoms usually occurs 18-36 hours after the toxic food is eaten but may not appear for up to 8 days (Kvenberg, 1991). The early indications of botulism intoxication are lassitude, weakness and vertigo followed by double vision and paralysis of the neck muscles which cause difficulty in speech and swallowing. Symptoms become more severe and progress downward through the body. Eventually, the victim has difficulty breathing because of paralysis of the diaphragm and chest muscles, and must be ventilated to breathe or death from asphyxia will result. The toxic dose of botulism toxin is very small. A few nanograms of the toxin are sufficient to cause symptoms. Botulinal toxin causes severe and prolonged neuromotor impairment that may be permanent (Kvenberg, 1991). It is for this reason that antitoxin should be administered quickly to neutralize the effect.

C. botulinum is ubiquitous in the natural environment and can be found in ocean sediments as well as in animal, bird and fish intestines. However, it is found mainly in soils and sediments. It is described as a soil organism and the types of *C. botulinum* found vary, depending on location. *C. botulinum* type E has been frequently shown as a contaminant of seafood and is primarily of marine origin. This type has been isolated from a number of coastal areas and from various seafood species (Eklund and Poysky, 1965, 1967; Nickerson *et al.*, 1967). The types of botulism are divided quite well geographically. Type A botulism is the main cause of outbreaks in the western United States, China and Argentina. Type B botulism is associated with outbreaks in Europe, while type E botulism occurs in northern Japan, Alaska, Canada, regions of Russia and Iran. The reasons for this distribution are not well understood. However, type A grows well in neutral to alkaline soil with low organic content, which explains its absence in the highly cultivated soils of the eastern United States and Europe. Type E is found in northern regions due to its tolerance for low temperatures.

1.5.4.1. Factors influencing growth of *C. botulinum*

The nutritional requirements for *C. botulinum* are complex and include several amino acids, growth factors and mineral salts. The organism grows best in rich organic media, such as meat. However, in the absence of competing microorganisms, it can grow in relatively poor media, such as fruits and vegetables (Sofos *et al.*, 1979). It is therefore important to consider the growth of *C. botulinum* in any food unless inhibitory factors are present. The factors affecting the growth of *C. botulinum* and toxin production in foods are temperatures, pH, a_w, gas atmosphere, food additives and the background microflora. The organism responds differently to each factor, and a combination of factors is more inhibitory than one factor alone.

Proteolytic strains of *C. botulinum* have a minimum growth temperature of 10°C, while the lower limit for non-proteolytic strains is 3.0°C in optimal growth conditions (Hauschild, 1989; Graham *et al.*, 1997). The upper temperature limit for proteolytic strains is between 45-50°C, and their optimum growth temperature is ~40°C. Non-proteolytic strains have their upper temperature limit of ~40°C and an optimum growth temperature of ~30°C. *C. botulinum* type E spores have been shown to germinate rapidly at 50°C (Grecz and Arvay, 1982).

The minimum pH for growth of proteolytic strains of *C. botulinum* ranges from 4.6-5.0, while the minimum pH for growth of non-proteolytic strains is ~5.0 (Hauschild, 1989). However, it was discovered that pH alone was not a good indicator of growth and toxin production, particularly in high protein substrates. The upper pH limit for growth and toxin production by *C. botulinum* was reported to be between pH 8-9.

Water activity (a_w) plays a key role in the growth of all microorganisms, and the minimum inhibitory a_w is dependent on the type of solute. For foods or media containing NaCl, the minimum a_w for growth of *C. botulinum* is 0.94-0.96 for types A and B, and 0.97

for type E (Baird-Parker and Freame, 1967). Similar results were obtained if KCl, glucose and sucrose were used as humectants. Glycerol, on the other hand, reduced the minimum a_w by up to 0.03 units. For types A and B, the limiting a_w is then 0.93, while for type E, it was 0.95. *C. botulinum* spores are capable of germinating at a_w levels as low as 0.89 under otherwise optimal conditions.

Although *C. botulinum* is classified as an anaerobic microorganism, it is possible for its growth to occur in foods that are exposed to oxygen. This is due to the redox potential (E_h), which is related to the oxygen concentration present within the food or the media. A high E_h is due to the presence of oxygen. *C. botulinum* cannot grow in foods with an E_h above +30-150 mV, however spores are capable of germinating at an E_h of +414 mV. If other inhibitory factors are present, the upper E_h level for growth would be lowered. Sperber (1982) showed that most foods exposed to oxygen have an E_h low enough to support the growth of *C. botulinum*. Many studies have shown the ability of *C. botulinum* to produce toxin in both aerobic and anaerobic conditions (Christiansen and Foster, 1965; Ajmal, 1968; Sugiyama and Yang, 1975). The use of various gas atmospheres has been found to prevent, promote or have no effect on *C. botulinum* spores, depending on the various gas mixtures.

1.5.4.2. *C. botulinum* in food

C. botulinum was first recognized and associated with food poisoning by van Ermengem in the 1890's, and since then, there have been over 16,000 cases of botulism, with more than 2,700 fatalities (McClure *et al.*, 1994). *C. botulinum* has been associated with a variety of food products, including fish, meat, poultry, fruits and vegetables. Outbreaks have also occurred in other products such as cheese. Botulism was originally only associated with meat products, as denoted by its name which is derived from '*botulus*', the Latin word for sausage. However, its occurrence in meats is rare compared to its occurrence in fish, and in fruits and vegetables. It is widespread in the environment and thus increasing its probability

of being found in the food supply. Although the incidence of *C. botulinum* in the food supply is relatively low, it is recognized as a serious public health hazard due to its high mortality rate.

The level of contamination in meat and meat products by *C. botulinum* is relatively low compared to fish and fish products. This is due to the fact that contamination on the farm is lower than in aquatic environments. It has been shown that animals themselves can be carriers of *C. botulinum* spores, which would result in internal contamination of meat (Lucke and Roberts, 1993). Nearly all toxin types isolated from meat are either type A or B.

C. botulinum contamination in fish occurs as a result of exposure both before the fish is harvested or during processing. The contamination before harvest is due to the fact that *C. botulinum* spores are found frequently in the aquatic environment of the fish. *C. botulinum* type E is the most prevalent type of *C. botulinum* found in the environment except in southern California, where type A predominates (Peltroy *et al.*, 1982). The majority of botulism cases involve *C. botulinum* type E spores. Seafood held under refrigeration temperatures above 3.0°C can support the growth of type E organisms (Schmidt *et al.*, 1961). Since *C. botulinum* type E can grow at low temperatures, refrigerated storage is not a barrier to its outgrowth and may, in fact, enhance the competitive capability of this strain.

The level of contamination of *C. botulinum* in fruits and vegetables may vary due to different agricultural practices, such as the use of manure fertilizer. Fruits and vegetables harvested from the soil are more susceptible to contamination. Products associated with *C. botulinum* include asparagus, bean, carrot, celery, corn, lima bean, olive, potato, turnip, apricot, cherries and peach. Approximately equal numbers of *C. botulinum* type A and B spores were detected (Meyer and Dubovsky, 1922).

The incidence of *C. botulinum* spores in dairy products is very low (<1 spore/liter) (Collins-Thompson and Wood, 1993). Although the number of botulism outbreaks in

cheeses is also low, there is concern since the reduced fat, high moisture and low salt cheeses could support the growth of *C. botulinum*. There has been very little work done to determine the level of contamination of dairy products.

The presence of *C. botulinum* spores in food is not of great concern to adults since the intestine of adult is already colonized with the normal digestive microflora, which prevent *C. botulinum* spores from germinating and subsequently producing toxin. However, spores in infant foods pose a greater hazard because the spores are able to colonize the infant's intestine, particularly those less than 1 year of age, to produce toxin and cause infant botulism. Honey is of primarily concern, since it may contain *C. botulinum* spores. Type A spores were most common but type B, C and D were also found in a few samples (Sakaguchi, 1979).

The largest problem worldwide remains foods preserved in the home, whether from home canning, home curing or home fermentation. The food product must receive a treatment that is lethal or at least inhibitory to vegetative bacteria cells, to allow successful competition, growth and toxin production of *C. botulinum*. Inadequate processing is the chief cause of this selective process and can happen in several ways. Low heat treatments that are intentional or accidental are the primary cause of this failure. For intoxication to develop, the product must be served without an adequate preparatory cooking step before eating since the preformed toxins are inactivated at 60°C within 5 minutes (Sakaguchi, 1979). A fermentation or acidification process that operates at a pH range above 4.6 or a brining process with low salt levels also can allow survival and growth of *C. botulinum*. With these conditions in place, the surviving spores then germinate and multiply given the proper circumstances, ultimately making the product toxic.

1.5.4.3. Control of *C. botulinum* in food

The majority of food preservation techniques were designed with *C. botulinum* in mind. Control of *C. botulinum* implies control of other pathogenic organisms present in the food product since it is the most heat resistant pathogenic organism. In most cases, *C. botulinum* is controlled by inhibition rather than destruction (Hauschild, 1989). There are a number of ways to prevent the germination of *C. botulinum* spores, and they are dependent on the type of food product. Methods to control the growth of *C. botulinum* in foods include thermal processing, low temperature preservation, irradiation, nitrites and reduced a_w.

Thermal processing is the most common method of food preservation. The process is designed to kill *C. botulinum* spores of group I, i.e., proteolytic strains, since those are the most heat resistant. The thermal death time (D-value) required to eliminate 90% of the population at a given temperature varies among different strains of *C. botulinum*. Type A and B spores are the most heat resistant, and have $D_{121}{}^{\circ}{}_{C}$ values in the range of 0.1 to 0.2 minute. This D-value of 0.2 minute at 121°C has been adopted as a standard for the food industry, and is used when calculating thermal processes. The z-value, which is the temperature change necessary to bring about a tenfold change in the D-value, is ~10°C and varies slightly among strains. Group II *C. botulinum* strains, i.e., non-proteolytic strains, are less heat resistant than group I strains. However, their survival in pasteurized, refrigerated food products or minimally processed food products are of concern because of their ability to grow at refrigeration temperatures (Dodds, 1994).

The resistance of botulinal spores to ionizing radiation has been studied extensively due to the promising effect of gamma irradiation sterilization. *C. botulinum* spores are one of the most radiation-resistant spores of public health concern (Dodds, 1994). D-values of proteolytic strains at -50°C to -10°C are in the range of 0.2-0.45 Mrads (2.0-4.5 kGy) (Dempster, 1985). In the broad range of -200°C to 50°C, D-values decrease progressively by ~1 krad/°C. Depending on the irradiation environment, D-values generally increase again

with increasing temperature, starting at about 20°C (Grecz *et al.*, 1971). It has been shown that *C. botulinum* type E spores are only slightly more sensitive to gamma irradiation than proteolytic strains of *C. botulinum*. The D-values for *C. botulinum* type E spores are between 0.1-0.2 Mrad (Erdman *et al.*, 1961).

Nitrite is the most common preservative used to control the growth of *C. botulinum*. It is capable of inhibiting spore germination in certain specific laboratory media, but at levels permitted in food, it does not entirely inhibit spore germination. The exact mechanism of botulinal inhibition by nitrite is still unknown, however, nitrite acts by reacting with iron-sulphur compounds, such as ferridoxin, to produce iron-nitric oxide complexes. These complexes in turn interfere with energy metabolisms. Nitrite also inhibits the phosphoroclastic system, which involves conversion of pyruvate to acetyl phosphate, electron transfer and ATP formation (Hauschild, 1989). Nitrite delays but does not prevent botulinal outgrowth and toxin production in food products. The effectiveness of nitrite depends primarily on the depletion rate of nitrite, as well as the death rate of the spores that have germinated (Christiansen, 1980). The depletion rate is dependant on product formulation, pH, time and temperature during processing and storage (Fox and Nicholas, 1974). Other preservatives employed to control the growth of *C. botulinum* in foods include sorbic acid, parabens, phenolic antioxidants, polyphosphate, ascorbates and smoke.

Bacteriocins have been shown to inhibit *C. botulinum* in a variety of food products. These bacteriocins come from a variety of sources, particularly lactic acid bacteria. Strains found to produce bacteriocins inhibitory to *C. botulinum* include *Pediococcus pentosaceous*, *Lactococcus lactis* subsp. *lactis*, *Lactobacillus acidophillus* and *Lactobacillus plantarum* (Okereke and Montville, 1991a,b). Nisin, a bacteriocin produced by *L. lactis* subsp. *lactis*, has been shown to be an effective anticlostridial agent in many foods, particularly dairy product (Hurst, 1981). However, the application of nisin to meat products as an antibotulinal agent is less promising since the activity of nisin increased at reduced pH levels. Nisin treatment of cod, herring and smoked mackerel fillets, inoculated with *C. botulinum* spores,

resulted in a delay in toxin production (Taylor *et al.*, 1990). However, in all samples, toxigenesis preceded spoilage. Subtilin, a bacteriocin produced by certain strains of *B. subtilis*, was also capable of inhibiting growth of *C. botulinum* (Jay, 1983). It is promising due to the fact that it is stable in acidic environments as well as being heat stable.

The growth of other microorganisms in the food may also prevent the growth of *C. botulinum* by changing the environment or by producing inhibitory substances. Lactic acid bacteria have been shown to produce acid, and inhibit *C. botulinum* in meat products (Tanaka *et al.*, 1980, 1985). Other inhibitory substances produced by *Bacillus licheniformis*, *C. perfringens* and *Enterococci* are also capable of inhibiting growth of *C. botulinum* (Wentz *et al.*, 1967; Smith, 1975; Kafel and Ayres, 1969).

1.6. Formulation of Value-Added Seafood Products

In North America, consumers are accustomed to the fillet type of texture in most of their fish products. Products made from minced fish, e.g., fish sticks have a softer texture but command a lower price than products made from fish fillets. However, high quality minced fish preparations have a tough, rubbery texture, which apart from its use in shellfish analogues, could be reformulated to appeal to North American taste. Due to its excellent water binding capacity, minced fish can be made into breaded and battered fish products. Mince of rainbow trout processing wastes or trimmings was used throughout the study.

1.7. Research Objectives

A local seafood producer Via-Mer (St-Hyacinthe, Quebec, Canada) has addressed concerns about the disposal of rainbow trout processing wastes/trimmings and demonstrated an interest in converting the otherwise wasted material into profitable value-added products. However, to date, few research groups have focused on the use of fish processing wastes to formulate value-added products. Furthermore, with increasing energy costs associated with freezing, which is the common storage method used for value-added food products, and consumer demands for "near-fresh" quality food products, MAP is considered a promising alternative in extending the shelf-life of the products. Consequently, it is essential to optimize the various storage and packaging environments without compromising the safety of food products.

The specific objectives of this research were as follows:

(1) To formulate good quality, consumer acceptable and commercially viable value-added products using rainbow trout processing wastes or trimmings.

(2) To determine the physical, chemical, microbiological and sensory changes of MAP value-added trout products.

(3) To determine the public health safety of MAP value-added trout products in challenge studies with *Listeria monocytogenes* and *Clostridium botulinum* type E.

CHAPTER 2:
FORMULATION OF VALUE-ADDED TROUT PRODUCTS

2.1. Introduction

Due to the increasing concerns about dumping of fish processing wastes and dwindling of fish stocks, the seafood processing industry is constantly searching for alternative uses of fish processing wastes and underutilized fish species. Interest in the recovery and utilization of minced fish flesh increased dramatically during the early 1970s (Babbitt, 1986). When fillets are prepared, pieces of prime quality fish flesh remain on the bone rack. These racks are available for further processing, specifically mincing. Production of minced fish flesh offers several advantages over the more conventional means of using seafood: (1) provides a higher yield; (2) permits use of filleting waste and nontraditional species; (3) easy incorporation with other foodstuffs to increase nutritional aspects and palatability; (4) cost is fairly minimal; and (5) it is a simple process requiring no special skills and a very short processing time (Bligh and Duclos-Rendell, 1986).

Value-addition is essentially the process of turning an otherwise wasted material into profitable food products. The unique texture-forming properties of minced fish make it an excellent base for manufacturing value-added seafood tailored to the requirements of a wide range of consumer interests. The convenience of value-added seafood products is gaining popularity in industrialized countries.

The objective of the initial phase of this study was to formulate good quality, consumer acceptable and commercially viable value-added trout products, using trout processing wastes or trout trimmings. The use of processing wastes/trimmings, instead of actual trout flesh, could be a tremendous economic advantage to the seafood processing industry since it utilizes low cost raw material and addresses concerns about the disposal of processing waste.

2.2. Materials and Methods

2.2.1. Materials used in Product Formulation

The main ingredient in the formulation of value-added trout products was trout mince. Trout mince was made from trout processing wastes or trimmings, and was obtained from a local seafood producer, Via-Mer, St-Hyacinthe, Quebec, Canada. Whole trout was first deheaded and gutted. After filleting, the frames and trimmings were passed through a deboning machine to remove bones, skin and connective tissues. Trout mince was then obtained and frozen until use. The unit operations in the production of trout mince are shown in Figure 2.1. All other ingredients used in the formulation of the value-added trout products (Table 2.1) were obtained from local supermarkets, with the exception of spice mix which was obtained from McCormick Canada Inc., Mississauga, Ontario, Canada (code: 97361-00).

2.2.2. Formulation of Value-Added Trout Products

A total of seven different formulations were made: trout balls, trout bites, trout burgers, trout croquettes, trout pancakes, trout puffs and trout wontons. The recipes for each formulation were obtained from several cookbooks (TIME-LIFE Books, 1979; Howarth, 1983; Canadian Department of Fisheries, 1959; Punyasingh, 1992). These recipes were selected on the basis of their (1) ease of preparation, (2) suitability for large scale production using available equipment, and (3) appeal to both North American and Asian tastes. They were modified to use trout mince as the main ingredient while the spice mix (McCormick Canada Inc.) was used as a substitute for most spices in the recipes. The appropriate levels of the various ingredients used in these formulations are summarized in Table 2.2.

Trout mince was defrosted before mixing. The appropriate amounts of trout mince and all other ingredients for each formulation were mixed together until blended uniformly. The actual levels of the various ingredients used in the production of the various value-added trout products are shown in Table 2.2. Each formulation was formed into its desired shape and coated when necessary with either breadcrumbs, crushed cornflakes or wonton covers (Table 2.3). All formulations were deep fried in vegetable oil at 140°C for 2-8 min (Table 2.3), with the exception of trout pancakes which were pan-fried, to give products a golden brown color. Cooked products were then placed on paper towels and drained of excess oil for 2 min prior to sensory evaluation.

2.2.3. Evaluation Procedures

All prepared, fully cooked value-added trout products were evaluated by 20, non-smoking untrained panelists in a sensory evaluation room. Panelists were asked to assess the overall quality of each product based on product appearance, odor and taste and to vote "like" or "dislike" for each product. All products were evaluated in a random manner and panelists were asked to rinse their mouth with water prior to testing each product.

2.2.4. Proximate Analysis

A complete proximate analysis and the caloric value of trout burgers and trout wontons was performed by Bio-Lalonde Laboratories (Division of SGS Canada Inc., Pointe-Claire, Quebec, Canada).

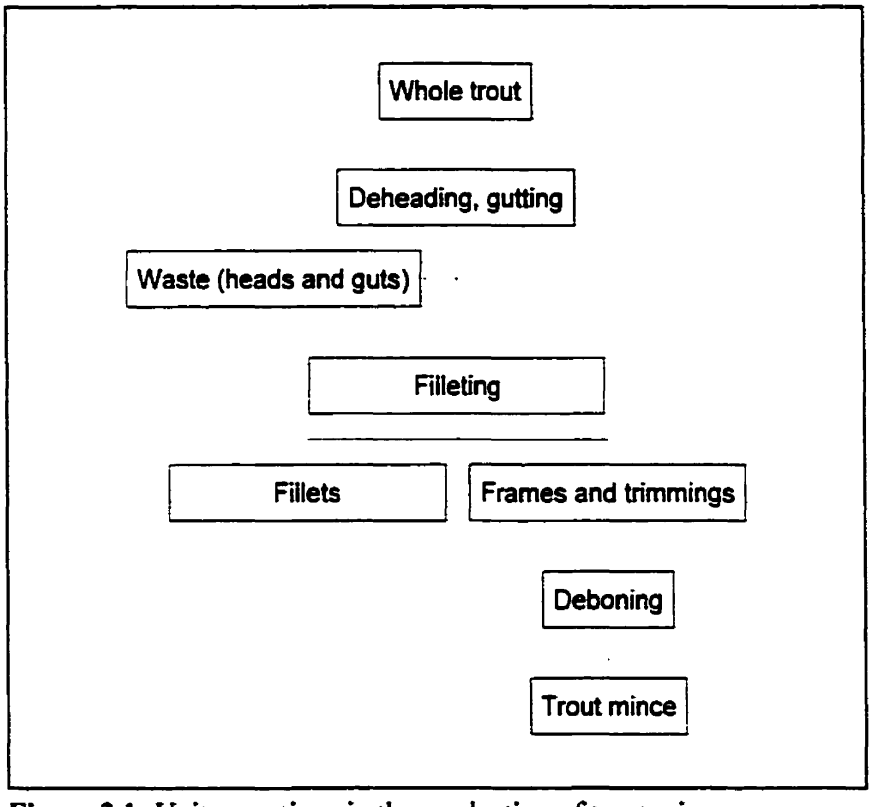

Figure 2.1: Unit operations in the production of trout mince.

Table 2.1: Ingredients obtained from local supermarkets and used in the formulation of value-added trout products.

Ingredient	Company
Cayenne pepper	McCormick Canada Inc., London, Ontario
Cornflakes	Kellogg Canada Inc., Etobicoke, Ontario
Dehydrated onion flakes	McCormick Canada Inc., London, Ontario
Egg	Briska Inc., Montreal, Quebec
Fresh onion	Etheir & Freres Ltee, Mirabel, Quebec
Fresh potatoes	Provigo Distribution Inc., Montreal, Quebec
Garlic powder	McCormick Canada Inc., London, Ontario
Ground white pepper	McCormick Canada Inc., London, Ontario
Homogenized milk (3.25%)	Parmalat Canada, Montreal, Quebec
Instant mashed potato	General Mills Canada Inc., Mississauga, Ontario
Lemon juice from concentrate	Borden Foods Canada, Etobicoke, Ontario
Mild cheddar cheese	Kraft Canada Inc., Don Mills, Ontario
Minute oats	Robin Hood Multifoods Inc., Markham, Ontario
Parmesan grated cheese	Kraft Canada Inc., Don Mills, Ontario
Plain breadcrumbs	Pastene Inc., Montreal, Quebec
Soya sauce	V-H Foods, Laval, Quebec
Table salt	The Canadian Salt Company Ltd., Pointe-Claire, Quebec
Vegetable oil (canola)	Procter & Gamble Inc., Toronto, Ontario
White all purpose flour	Les Cuisines Five Roses Kitchens, Montreal, Quebec
Wonton covers	Les Aliments Wong Wing Foods Inc., Montreal, Quebec

Table 2.2: Levels of various product ingredients (grams) used in the mixtures for individual formulations.

Ingredient	Trout balls	Trout bites	Trout burgers & wontons	Trout croquettes	Trout pancakes	Trout puffs
Trout mince	500	350	500	500	550	400
Breadcrumbs	275	-	-	-	-	-
Cayenne pepper	0.1	-	0.4	-	-	-
Cheddar cheese	-	-	-	60	-	-
Dehydrated onion flakes	-	-	50	-	-	-
Egg (beaten)	100	-	50	50	100	50/50*
Flour	-	20	-	-	22	-
Fresh onion (chopped)	-	-	-	30	100	-
Fresh potatoes (grated)	-	-	-	-	500	-
Garlic powder	4	-	16	-	-	1
Lemon juice	-	-	-	30	-	-
Mashed potato	250	-	-	120	-	350
Milk	-	50	-	-	-	-
Oats	-	-	100	-	-	-
Parmesan cheese	55	-	-	-	-	-
Salt	2	5	-	5	12	15
Soya sauce	-	2	28	-	-	-
Spice mix	-	15	50	25	25	25
Vegetable oil (canola)	-	11	-	23	-	-
White pepper	0.5	-	3	-	-	0.4

* 50 g of egg yolk and egg white (beaten stiff) each.

Table: 2.3: Specifications of various trout product formulations.

Specification	Trout balls	Trout bites	Trout burgers[2]	Trout croquettes	Trout pancakes	Trout puffs[3]	Trout wontons[4]
Weight of mixture (g)	10	10	75	20	40	30	10
Shape	Ball	Ball	Patty	Patty	Patty	Patty	NA
Batter[1]	-	-	+	-	-	-	-
Coating	No	Breadcrumbs	Cornflakes	Breadcrumbs	No	No	Wonton cover
Cooking method	Deep fry	Deep fry	Deep fry	Deep fry	Pan-fry	Deep fry	Deep fry
Cooking time (min)	2	2	5	2	3 (each side)	3	8

NA: Not applicable

[1] Mixture of 50 g egg, 75 g flour and 120 g water.

[2] Trout burgers were dipped into batter before coating with thin layer of crushed cornflakes.

[3] Beaten egg white was folded into the mixture after mixing all other ingredients.

[4] Edges of wonton cover were folded together with mixture placed in the center of the wonton cover.

2.3. Results and Discussions

2.3.1. Formulation of Value-Added Trout Products

This research investigated the potential use of seafood processing waste as a source of raw material for consumer acceptable products. The study was aimed at using rainbow trout processing wastes for the development of value-added products. Photographs of the 7 different formulations made: trout balls, trout bites, trout burgers, trout croquettes, trout pancakes, trout puffs and trout wontons are shown in Figures 2.2 to 2.8. All formulations could be easily made from readily available ingredients and formed using standard equipment for mass production. These products were designed to be heat and serve, convenient ready-to-eat meal solutions and snack food products. All products could be prepared within 10 minutes from refrigerator to table and could be cooked in the oven, microwaved, deep fried or pan-fried. The quality of reheated products after freezing remained similar to freshly made products.

2.3.2. Product Evaluation

The results of product evaluation are summarized in Table 2.4. It is evident from Table 2.4 that the majority of panelists like these products in the following order: wontons > burgers > croquettes/pancakes > puffs > bites > balls.

Trout balls had a nice appearance but the mashed potato in the recipe resulted in a soft texture, which was considered unacceptable by most of the panelists. The breadcrumb coating on trout bites gave them a nice mouth feel. However, the products did not have an attractive appearance and they were bland in flavor. The texture of trout puffs was regarded as too soft and the taste was too salty. Their soft texture was attributed to the folding in of the beaten egg white during formulation. Both trout croquettes and trout pancakes scored

highly (70% of panelists liked the products). Trout croquettes had a distinct taste because they contained cheddar cheese, which contributed to their high score. Trout pancakes were also highly acceptable with favorable appearance, odor and taste. The grated fresh potatoes used as one of the main ingredients provided a crunchy mouth feel to the product.

Out of all the formulations, trout burgers and trout wontons were the two which scored the highest with 85% and 95% of panelists voting "like" respectively. The same raw mixture was used for both trout burgers and trout wontons, nonetheless, the finished products, after additional coating and subsequent cooking, had their own distinct taste and uniqueness. Trout burgers had a firm meaty texture. Their highly rated taste was accompanied by the crispy crushed cornflakes coating. This combination made the trout burger a likely product to be used as a meat burger replacement. Trout wontons were rated the highest due to their appetizing odor, crispy wonton crust and tasty filling. The golden brown, flowery shaped finished products were very appealing to the panelists. Another favorable attribute was the convenience of the products for consumption. They could easily be reheated and served as snack food.

At present, there is no identifiable product found on the shelves of local supermarkets that resembles the formulated trout burgers and wontons. Neither seafood burgers nor seafood wontons are marketed. Similar value-added seafood products, such as battered and breaded seafood or fish sticks, are made from expensive raw materials, thus making these products fairly expensive. The cost of making both trout burgers and wontons was only ~$0.30/100 g of product. Hence, these products would not only provide the consumer with a wider range of choice but also be competitively priced when compared to similar available value-added fish products.

Table 2.4: Results of evaluation of value-added trout products prepared.

Value-added trout products	**Evaluation (%)**	
	"Like"	"Dislike"
Trout balls	40	60
Trout bites	50	50
Trout burgers	85	15
Trout croquettes	70	30
Trout pancakes	70	30
Trout puffs	65	35
Trout wontons	95	5

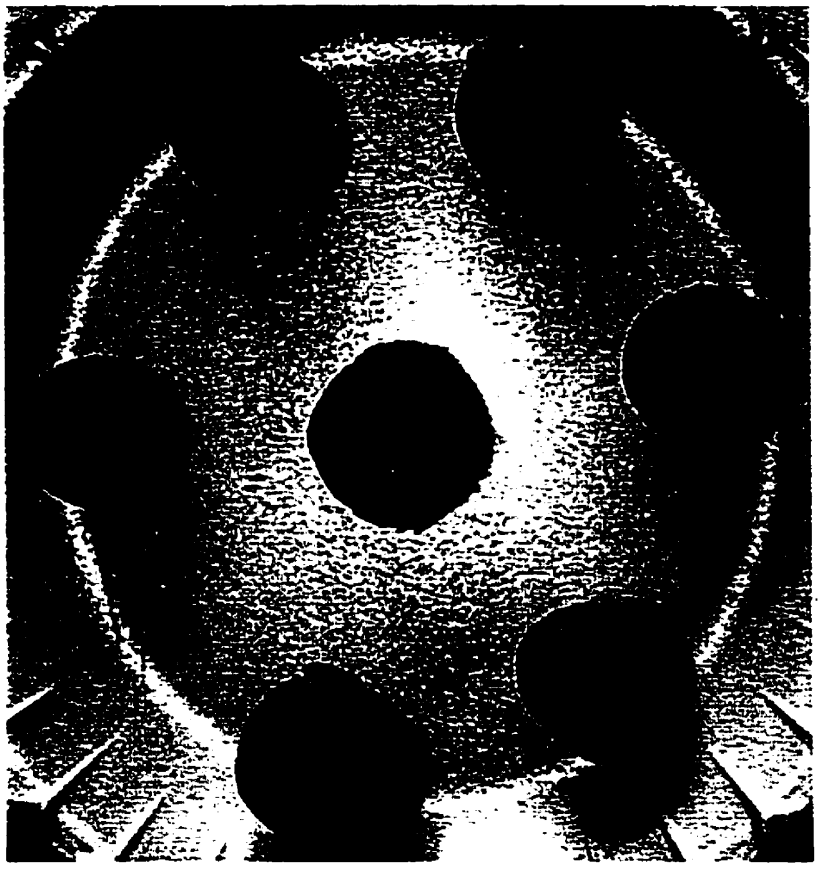

Figure 2.2: Photograph of trout balls.

Figure 2.3: Photograph of trout bites.

Figure 2.4: Photograph of trout burgers.

Figure 2.5: Photograph of trout croquettes.

Figure 2.6: Photograph of trout pancakes.

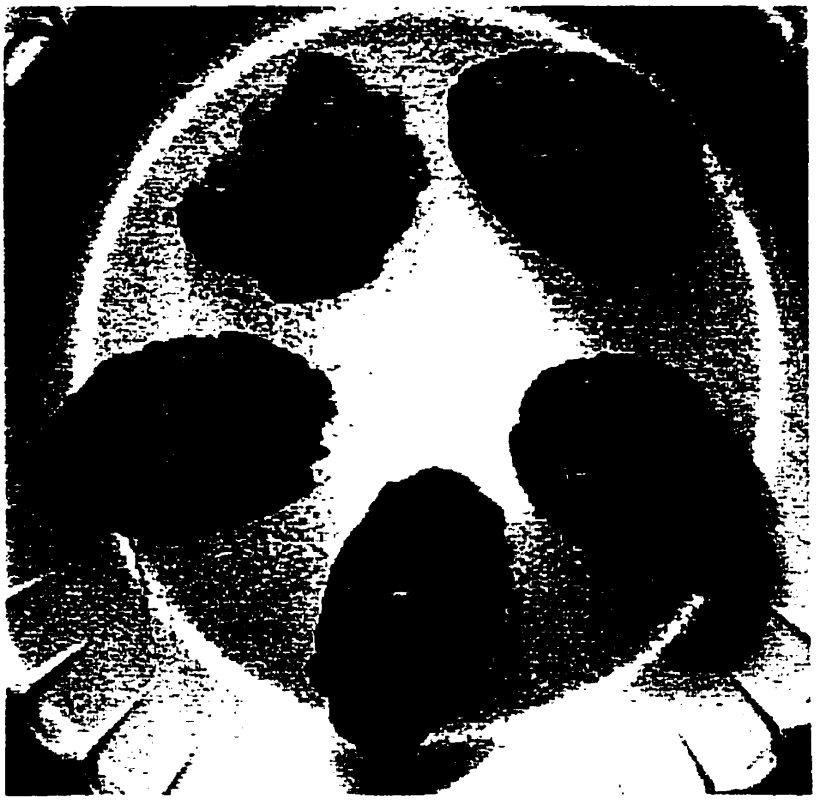

Figure 2.7: Photograph of trout puffs.

Figure 2.8: Photograph of trout wontons.

2.3.3. Proximate Analysis

The results of the proximate analysis on the most desirable products, i.e., trout burgers and wontons, and their caloric values are shown in Table 2.5.

Trout burgers and wontons had similar ash and protein contents, i.e., ~2%. However, these two products differed with respect to their moisture, carbohydrate and fat contents, as well as caloric values. Trout burgers tasted moist due to their higher moisture content than trout wontons. However, trout wontons had a higher carbohydrate and fat content compared to trout burgers. In fact, the fat content of trout wontons was approximately twice that of trout burgers. This was attributed to more oil being absorbed by the wontons' pastry cover compared to the burgers' crushed cornflake coating, resulting in wontons having a higher caloric value. However, the proximate analysis and caloric values of these products was similar to previous made value-added shrimp nuggets (Lyver, 1997) and to commercially available value-added seafood products.

Table 2.5: Results of proximate analysis on trout burgers and trout wontons (g/100 g of product).

Component	Trout burgers	Trout wontons
Moisture	40.1	18.3
Ash	2.9	1.9
Carbohydrates	29.6	40.8
Fat	13.5	27.5
Protein	13.9	11.5
Calories[1]	295.5	456.7

[1] Unit: calories/100 g product

2.4. Conclusions

This study has shown that good quality, consumer acceptable and commercially viable value-added trout products can be made using trout mince from trout processing waste. Sensory scores indicated that these formulated value-added products were highly acceptable and had tremendous market potential. The value-addition of seafood processing wastes could be a viable option to ensure the long term sustainable management of fishery resources, as well as reducing the volume of seafood processing waste produced by the seafood industry. Since trout burgers and trout wontons had the highest acceptability scores among panelists, these two value-added trout products were selected for subsequent shelf-life and safety studies.

CHAPTER 3:
SHELF-LIFE STUDIES

3.1. Introduction

A problem facing the seafood processing industry is the method of storage and distribution of products. To date, freezing is the main method of long term storage (>6 months) to ensure quality and to extend shelf-life of value-added food products. However, with increasing consumer demands for "fresh" products, and increasing energy costs associated with freezing and frozen storage, modified atmosphere packaging (MAP) is one of the most promising alternative methods for enhancing the shelf-life, quality, safety and marketability of food products while at the same time economizing the energy costs. Three commonly used MAP methods by the food industry to modify the gas atmosphere within a packaged product are vacuum packaging, gas packaging and the use of oxygen absorbent technology. However, the success of MAP technology depends on strict temperature control. Temperature abuse of MAP products could reduce shelf-life as well as compromising the safety of these products.

The objectives of this study were to (i) investigate the potential of MAP to extend the shelf-life of value-added trout products and (ii) to monitor the physical, chemical, microbiological and sensorial changes in these MAP products at refrigerated temperature (4°C) and mild temperature abuse (12°C) storage conditions.

3.2. Material and Methods

3.2.1. Preparation of Trout Burgers

The formulation of trout burgers was as described in Section 2.2.2. The trout burger mixture (75 g) was molded into a patty of 10.5 cm in diameter and 5 mm in height. The patty was then battered and coated with a thin layer of crushed cornflakes. Two types of trout burgers were made: partially cooked and fully cooked trout burgers. Partially cooked burgers were deep fried in vegetable oil at ~140°C for 2 min to set the batter, whereas fully cooked burgers were deep fried for 5 min. After cooking, the trout burgers were drained on paper towels to remove excess oil, and then cooled to room temperature (~21°C) prior to packaging. The total weight of each trout burger ranged from 110 to 120 g.

3.2.2. Preparation of Trout Wontons

The formulation of trout wontons was as described in Section 2.2.2. The trout wonton mixture (10 g) was placed on a 7.5 cm x 7 cm wonton cover. The edges of the cover were gathered and folded to wrap the filling in the center of the pastry. Edges of the wonton pastry were then lightly pressed to slightly seal the opening. Two types of trout wontons were made: raw and fried trout wontons. Raw wontons were uncooked. Fried wontons were deep fried in vegetable oil at ~140°C for 8 min. After cooking, the trout wontons were drained on paper towels to remove excess oil, and then cooled to room temperature (~21°C) prior to packaging. The total weight of individual trout wontons was ~18 g.

3.2.3. Packaging of Samples

Partially/fully cooked trout burgers (1 per bag) and raw/fried wontons (2 per bag) were packaged in 210 mm x 210 mm high gas barrier bags (OTR: 11.6 cc/m^2/day at 24°C, 0% relative humidity (RH); Cryovac Sealed Air Corporation, Mississauga, Ontario, Canada). Samples were packaged under the following conditions: air, air with an Ageless SS-200 oxygen absorbent (Mitsubishi Gas Chemical American Inc., New York, New York, United States), 60% and 80% CO_2 (balance N_2). Air packaged samples were sealed using an Impulse Heat Sealer. Ageless SS-200 oxygen absorbents were inserted into the appropriate bags which were then sealed as described previously. Gas packaging was done using a chamber type, heat seal packaging machine (Model A300/42, Multivac Inc., Kansas City, Missouri, United States). A Smith's proportional gas mixer (Model 299-028, Smith Equipment Division, Tescom Corporation, Minneapolis, Minnesota, United States) was used to give the desired proportions of O_2, CO_2 and N_2 in the package headspace. Duplicate samples of each packaging conditions were stored at 4 and 12°C and monitored for physical, chemical, microbiological and sensorial changes after 0, 3, 7, 14, 21 and 28 days until shelf-life of samples was terminated.

3.2.4. Headspace Gas Analysis

Samples were analyzed for headspace gas composition using a previously calibrated Servomex portable O_2/CO_2 analyzer (Model 1450B3, Servomex Company Inc., Norwood, Massachusetts, United States). Gas samples were withdrawn from each bag, through a silicone septum affixed to the exterior of the bag, with a gas-tight pressure-lock syringe and needle that was connected to the gas analyzer. Measurements were taken when gas samples were pumped through the analyzer.

3.2.5. Microbiological Analysis

Samples were evaluated for total aerobic plate count (APC), lactic acid bacteria (LAB), aerobic and anaerobic spore counts at each sampling time. For trout burgers, samples were aseptically cut in half. One half was used for microbiological testing and the other half was used for sensorial testing. Thirty grams of each half burger, and one wonton from each bag were aseptically transferred into a stomacher bag and 0.1% (w/v) sterile peptone water (Difco Laboratories, Detroit, Michigan, United States) added to give a 1:10 dilution. Each bag was stomached for 1 min in a Stomacher 400 LabBlender (A.J. Seward, Bury St Edmunds, United Kingdom). One ml of this suspension was aseptically transferred into 9 ml of 0.1% sterile peptone water, to give a 1:100 dilution. Subsequent dilutions were carried out as required to give countable plates. The media and the methodology used for enumerating the various types of microorganisms are summarized in Table 3.1. For all counts, 0.1 ml of the appropriate dilution was plated in duplicate using a spread plate technique. Plates were incubated aerobically with the exception of LAB and anaerobic spore counts which were incubated anaerobically using anaerobic Jars (BBL Microbiology Systems, Becton and Dickenson Co., Cockeysville, Maryland, United States). All plates were incubated at 35°C for 48 hours. Spores, both aerobic and anaerobic, were determined by first heat shocking the appropriate dilutions at 75°C for 20 min. Countable plates (30-300 colonies) were reported as $\log_{10}$ Colony Forming Unit per gram of sample (log CFU/g).

3.2.6. pH Measurement

The pH of each homogenized samples was measured using a previously calibrated (buffer solutions of pH 4 and 7, Fisher Scientific, Nepean, Ontario, Canada) Corning pH meter (Model 220, Corning Glass Works, Corning, New York, United States) with a gel filled polymer body combination electrode with Ag/AgCl reference (Model 13-620-104, Fisher Scientific, Montreal, Quebec, Canada). The electrode was inserted directly into the

suspension of stomached samples (1:10 dilution) after microbiological testing procedures were completed. Analysis (in duplicate) was carried out at each sampling time.

3.2.7. Sensory Analysis

All samples were evaluated subjectively for color, texture, odor and overall acceptability under fluorescent light at each sampling time. Each parameter of the sensory analyses was evaluated by 6 untrained panelists on coded samples in a random manner. A 7-point hedonic scale (7 = Extremely desirable, 1 = Extremely undesirable), described by Greer (1993) was used (Table 3.2). For each attribute, a score of <3.5 was considered to be unacceptable, implying that shelf-life was terminated.

Table 3.1: Outline of microbiological analysis performed on value-added trout products.

Organism	Media[1]	Supplier	Method	Incubation time (hour)
Mesophiles (APC)	TSA	Difco	Spread	48
Lactic acid bacteria	MRS	Difco	Spread	48
Aerobic spores	TSA	Difco	Spread	48
Anaerobic spores	TSA	Difco	Spread	48

[1] TSA: Tryptic Soy Agar; MRS: De Man, Rogosa and Sharp agar

Note: All samples were incubated at 35°C

Table 3.2: The 7-point hedonic sensory perception scale used in the study (Greer, 1993).

Score	Desirability
7	Extremely desirable
6	Desirable
5	Slightly desirable
4	Neither desirable nor undesirable
3	Slightly undesirable
2	Undesirable
1	Extremely undesirable

3.3. Results and Discussions

3.3.1. Headspace Gas Analysis

All samples showed similar trends in headspace gas changes, i.e., an increase in headspace CO_2 and a decrease in headspace O_2. The changes in headspace gas composition of the packaged samples are shown in Figures 3.1 to 3.16 respectively.

The level of headspace O_2 remained fairly constant (<5% decrease throughout storage) for most samples stored at 4°C (Figures 3.1-3.4), including air packaged samples. However, samples packaged with Ageless SS showed significant decrease in their package headspace O_2 to <1% within 3 days since Ageless SS is designed to lower O_2 in package headspace.

Headspace O_2 of air packaged samples stored at 12°C decreased to <5 % in 14 days for both partially and fully cooked trout burgers (Figures 3.5 and 3.6) while headspace CO_2 increased >20% within the same time period (Figure 3.13 and 3.14). A reduction in headspace O_2 and increase in CO_2 is mainly due to the growth of aerobic and facultatively anaerobic bacteria. Headspace O_2 of raw and fried wontons stored at 12°C remained fairly constant (Figures 3.7 and 3.8), including air packaged samples. Since the shelf-life of raw wonton was terminated within 7 days (details addressed in Section 3.3.2.), no significant headspace gas composition change was observed in such short period of time. However, headspace O_2 in fried wontons remained stable due to lack of microbial activity, which can be attributed to the cooking process it received.

Headspace CO_2 for samples stored at 4°C did not have significant changes throughout storage (Figures 3.9-3.12). The level of CO_2 in air and Ageless oxygen absorbent packaged samples stored at 4°C remained very low. There was slight decrease in headspace CO_2 in both 60 and 80% CO_2 gas packaged samples due to the dissolution of gaseous CO_2 into the

aqueous phase of the food products. The slight increase in headspace CO_2 toward the end of the storage mainly due to the growth and metabolism of both aerobic and anaerobic spoilage bacteria present in the product.

For samples stored at 12°C, the CO_2 level in packaged headspace remained stable with the exception of air packaged samples (Figures 3.13-3.16). The CO_2 level of air packaged partially and fully cooked trout burgers increased to ~35% at 14 and 21 days respectively (Figure 3.13 and 3.14). Similar trends were observed for both raw and fried trout wonton samples but with lower increases. The CO_2 level in raw and fried wontons increased to 11% (day 7) and 8% (day 21) respectively (Figure 3.15 and 3.16). An increase of CO_2 level in package headspace indicates microbial activity in the products.

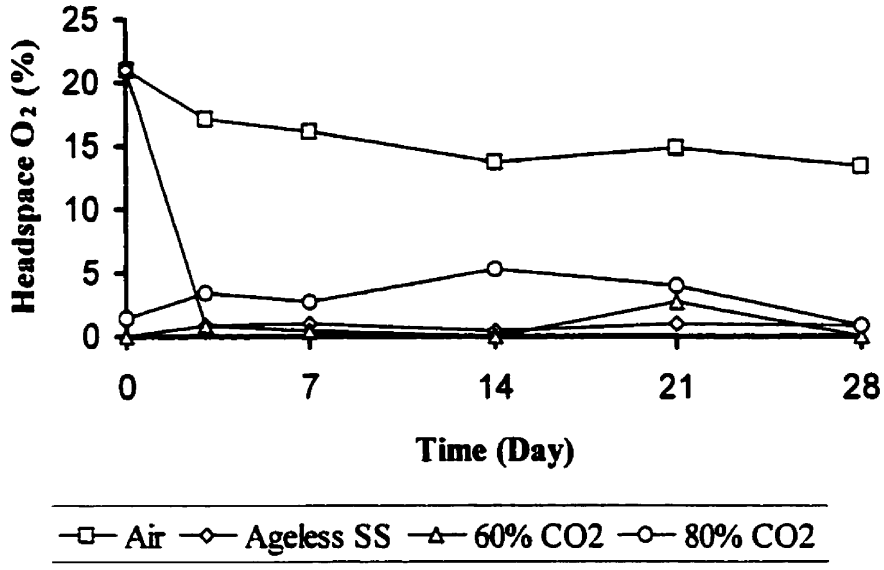

Figure 3.1: Changes in headspace O_2 of partially cooked burgers packaged under various gas atmospheres and stored at 4°C.

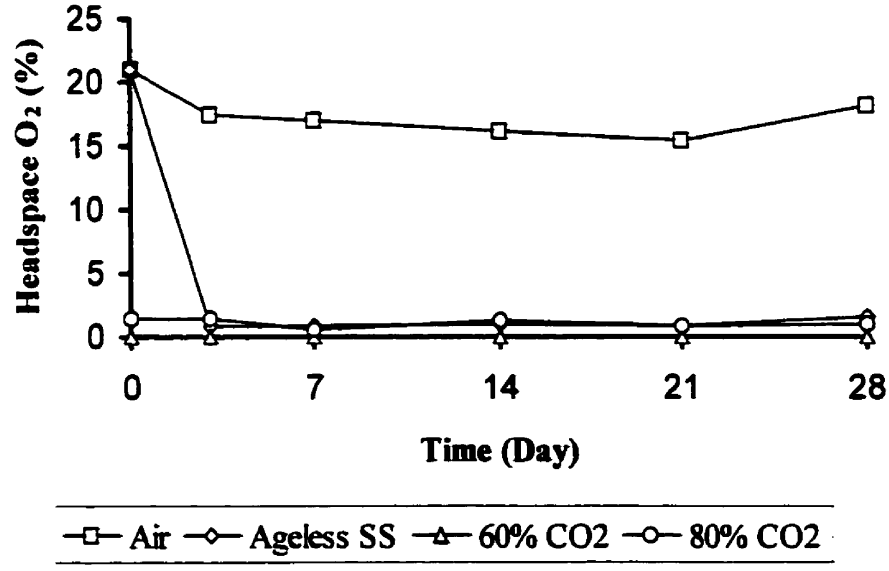

Figure 3.2: Changes in headspace O_2 of fully cooked burgers packaged under various gas atmospheres and stored at 4°C.

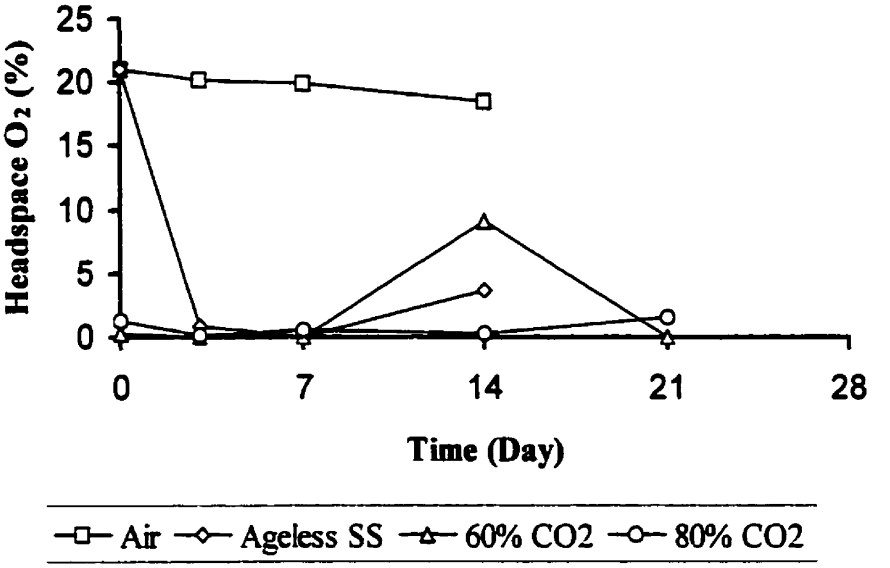

Figure 3.3: Changes in headspace O_2 of raw wontons packaged under various gas atmospheres and stored at 4°C.

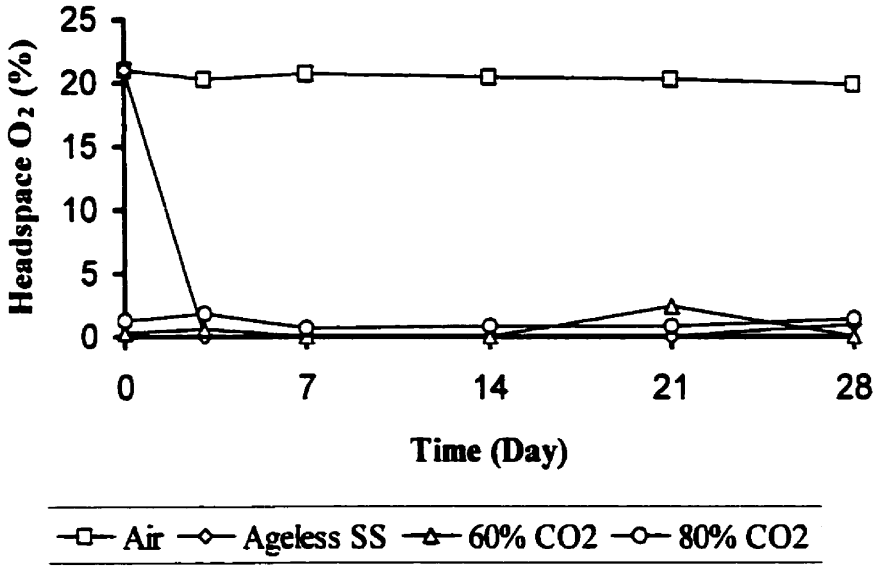

Figure 3.4: Changes in headspace O_2 of fried wontons packaged under various gas atmospheres and stored at 4°C.

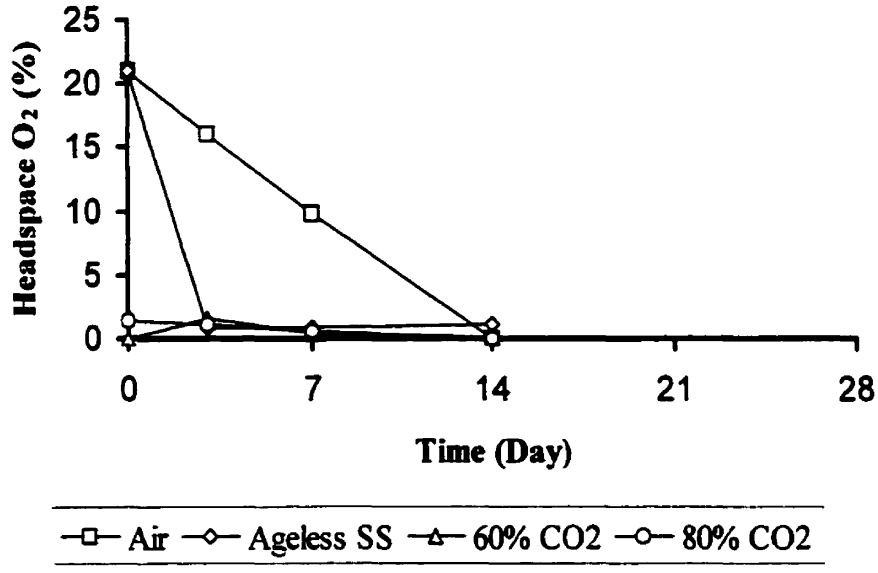

Figure 3.5: Changes in headspace O_2 of partially cooked burgers packaged under various gas atmospheres and stored at 12°C.

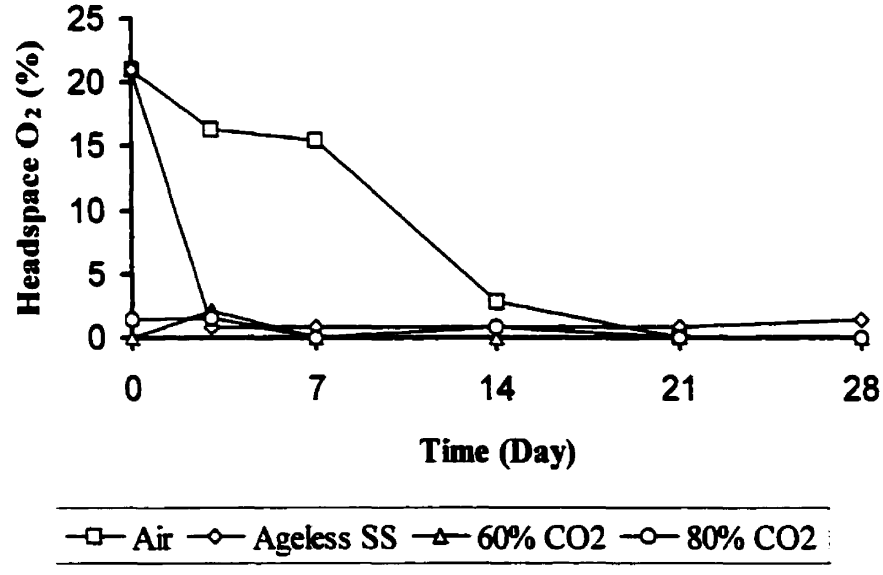

Figure 3.6: Changes in headspace O_2 of fully cooked burgers packaged under various gas atmospheres and stored at 12°C.

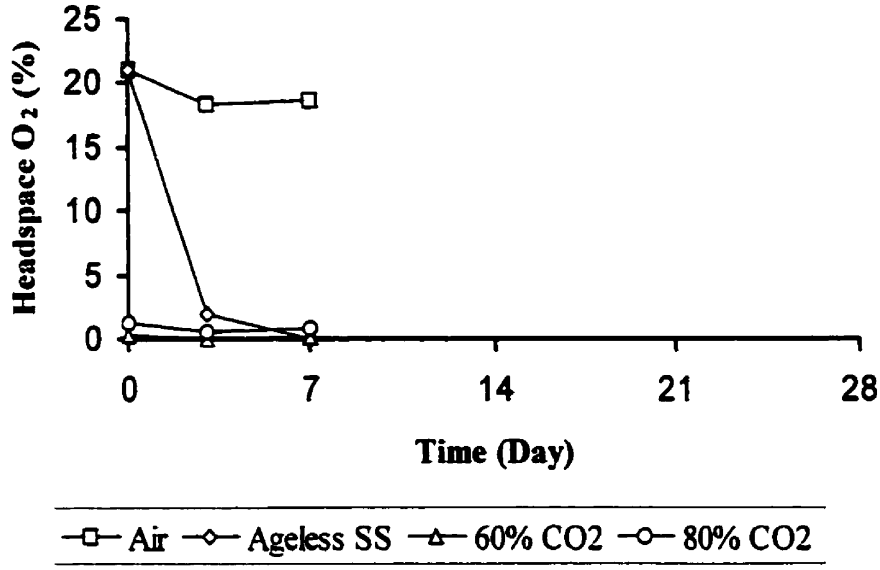

Figure 3.7: Changes in headspace O_2 of raw wontons packaged under various gas atmospheres and stored at 12°C.

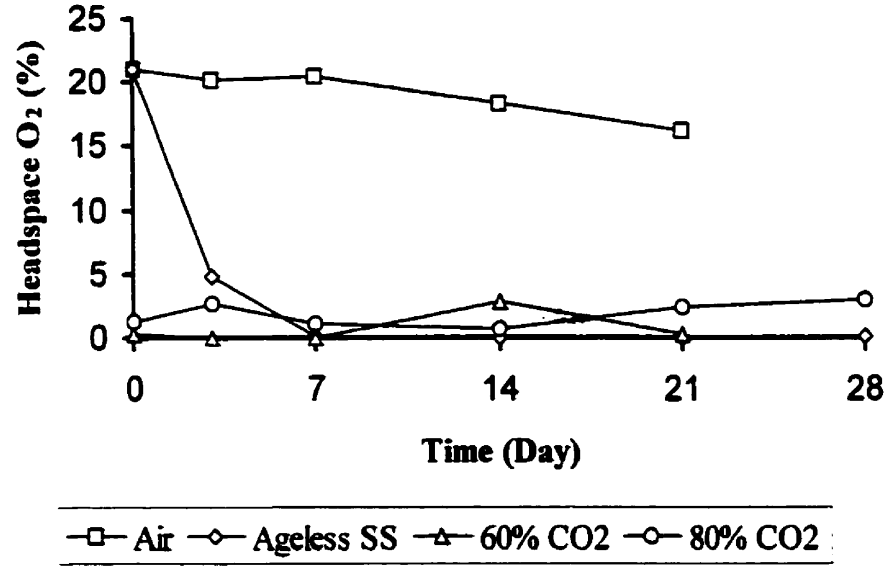

Figure 3.8: Changes in headspace O_2 of fried wontons packaged under various gas atmospheres and stored at 12°C.

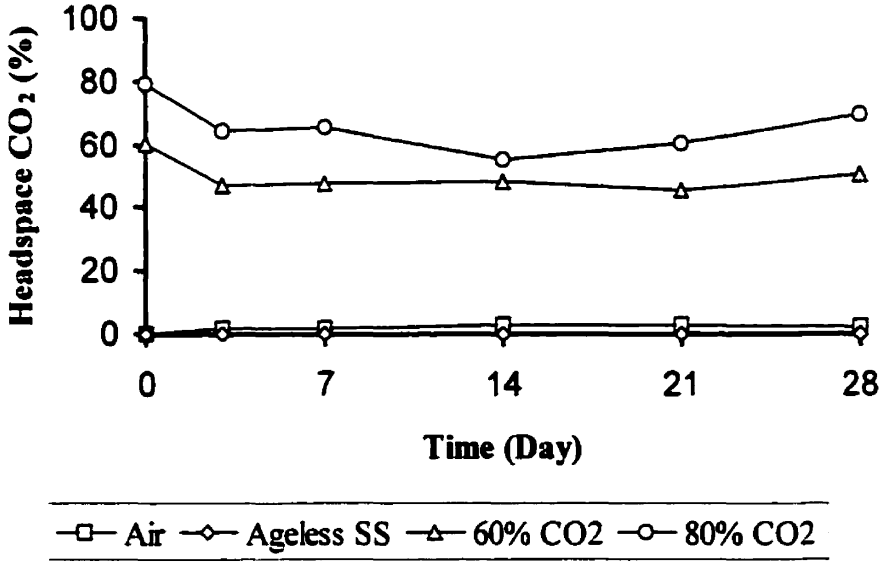

Figure 3.9: Changes in headspace CO_2 of partially cooked burgers packaged under various gas atmospheres and stored at 4°C.

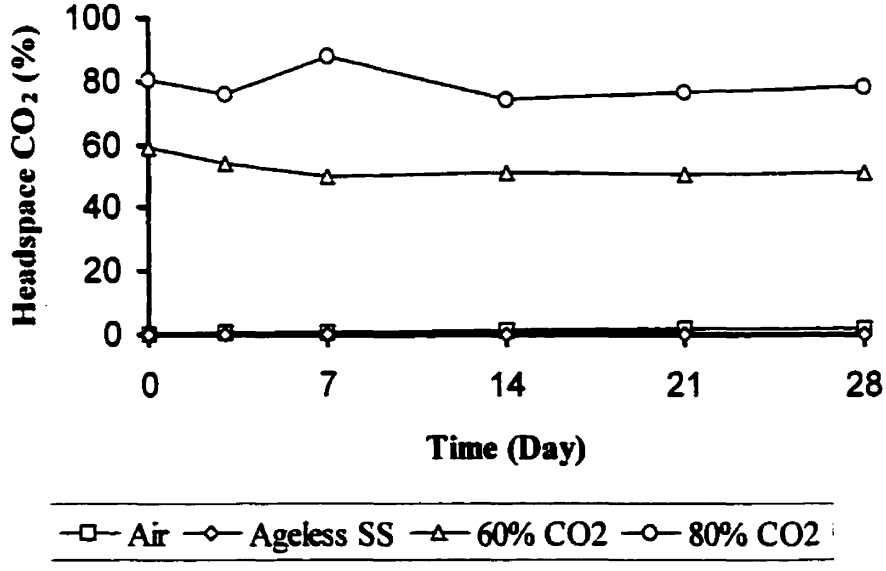

Figure 3.10: Changes in headspace CO_2 of fully cooked burgers packaged under various gas atmospheres and stored at 4°C.

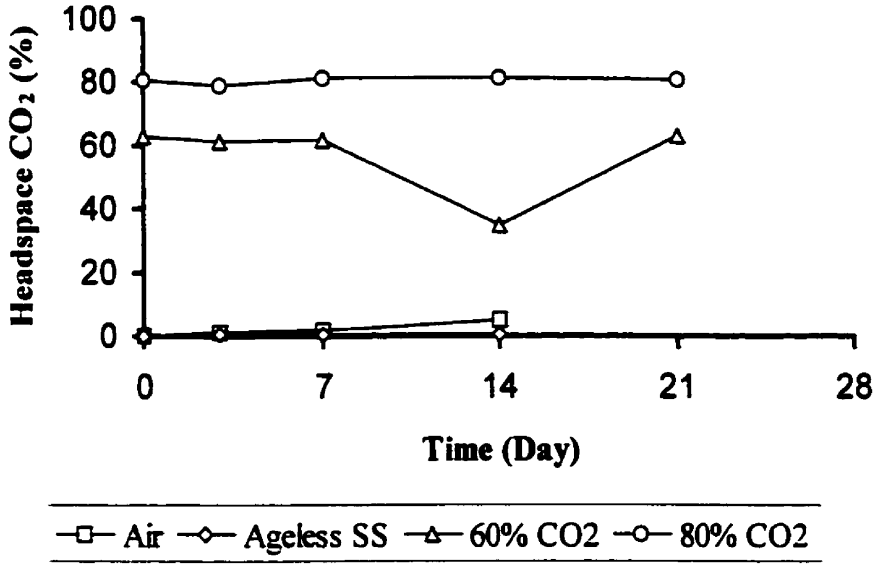

Figure 3.11: Changes in headspace CO_2 of raw wontons packaged under various gas atmospheres and stored at 4°C.

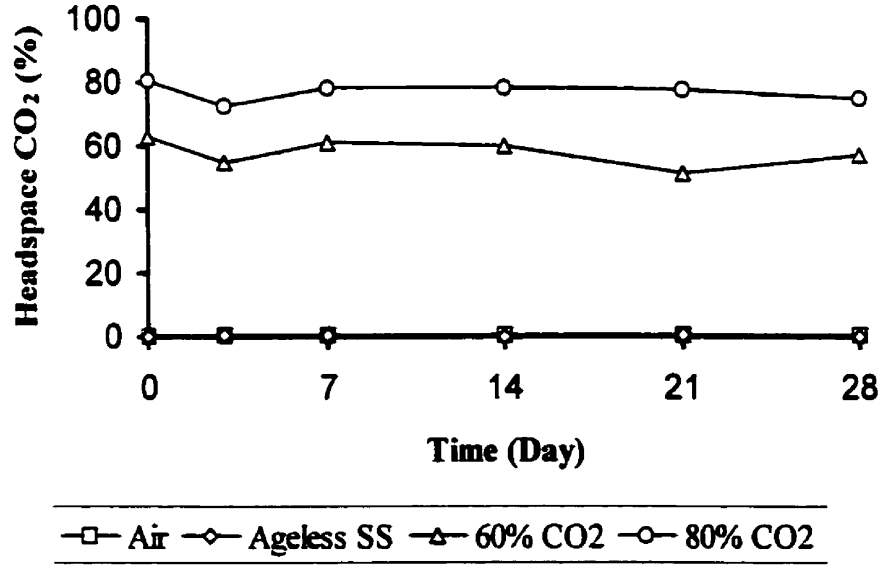

Figure 3.12: Changes in headspace CO_2 of fried wontons packaged under various gas atmospheres and stored at 4°C.

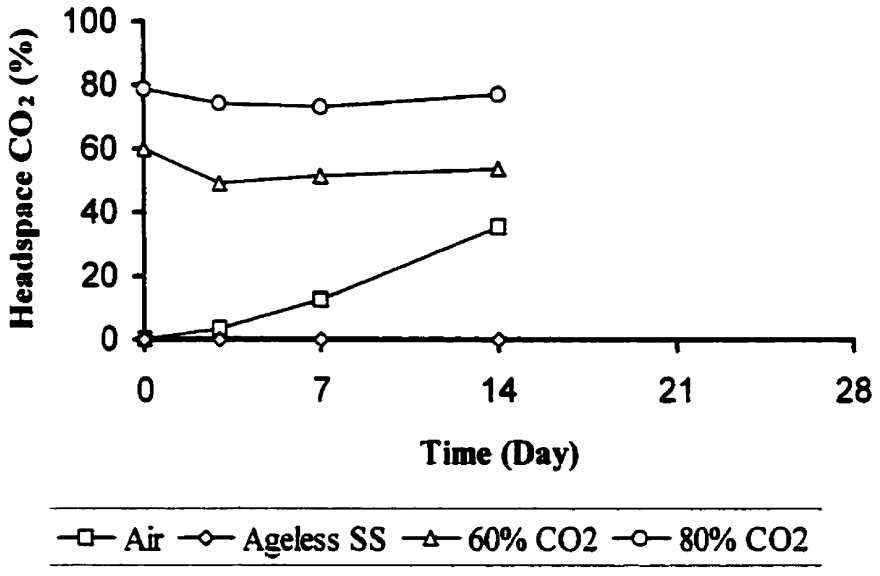

Figure 3.13: Changes in headspace CO_2 of partially cooked burgers packaged under various gas atmospheres and stored at 12°C.

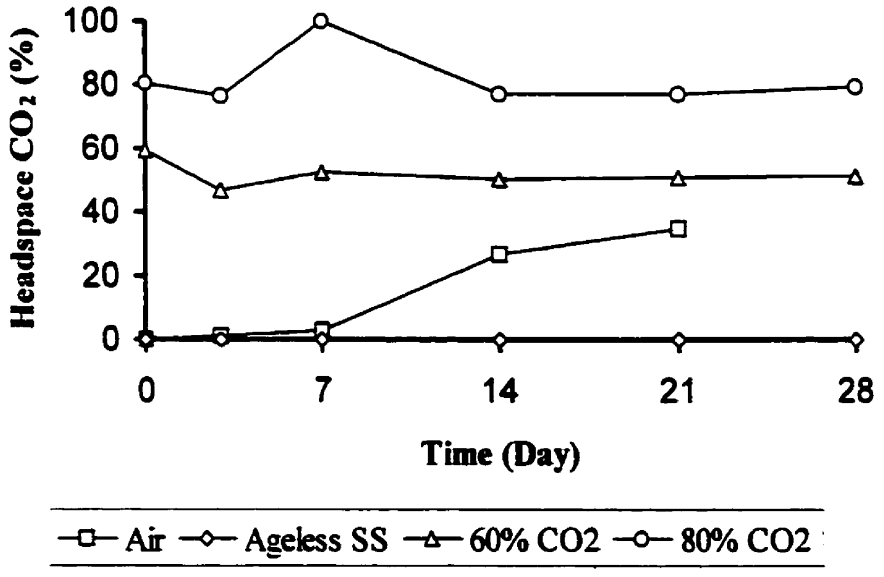

Figure 3.14: Changes in headspace CO_2 of fully cooked burgers packaged under various gas atmospheres and stored at 12°C.

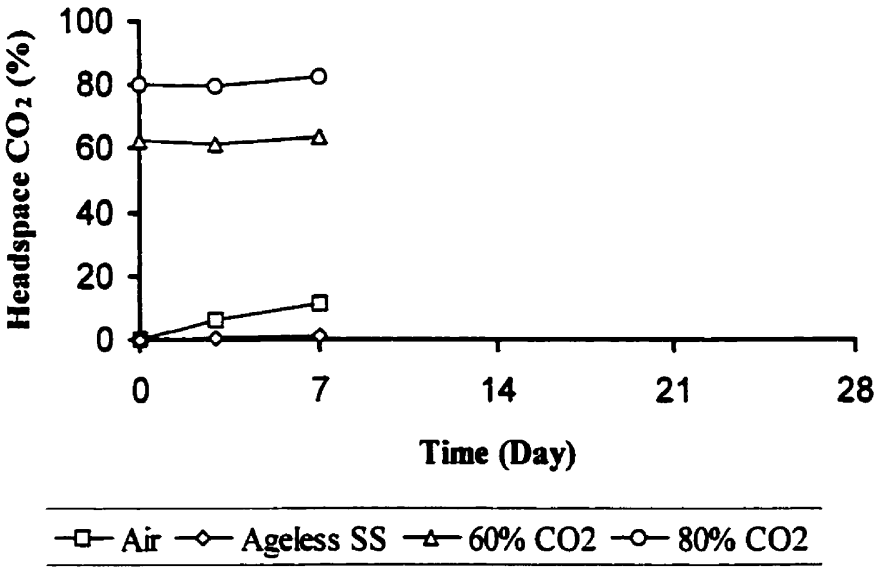

Figure 3.15: Changes in headspace CO_2 of raw wontons packaged under various gas atmospheres and stored at 12°C.

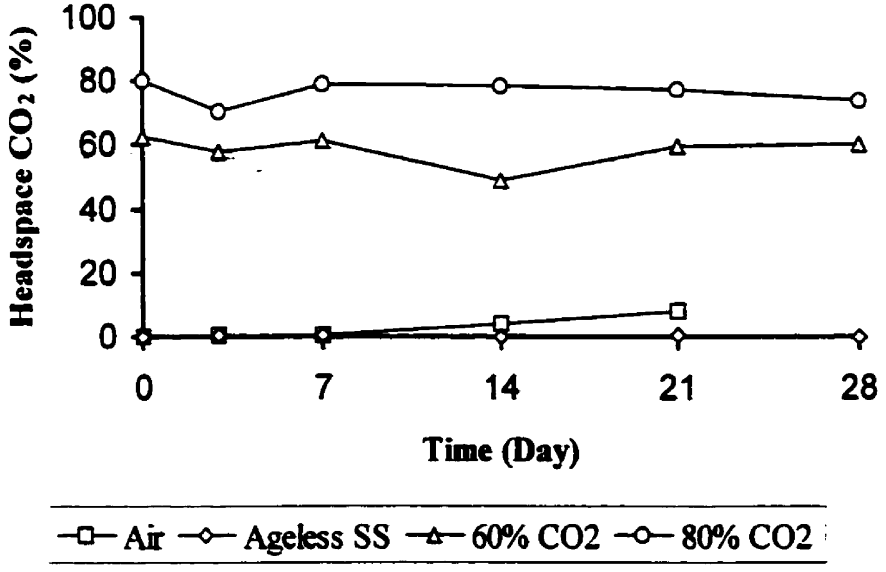

Figure 3.16: Changes in headspace CO_2 of fried wontons packaged under various gas atmospheres and stored at 12°C.

3.3.2. Microbiological Analysis

The microbiological changes of value-added trout products with respect to total aerobic plate count (APC), lactic acid bacteria (LAB), aerobic and anaerobic spores are shown in Figures 3.17-3.30.

3.3.2.1. Aerobic plate count (APC)

A total APC of approximately 10^7 CFU/g of bacteria in meat and muscle foods is considered to be the upper limit of microbiological acceptability (Niemand *et al.*, 1981). Hence, shelf-life of products was terminated when their APC reached 10^7 CFU/g.

The APC results for trout burgers and wontons stored at 4 and 12°C are shown in Figures 3.17-3.23 respectively. Most packaged products had an initial count of 10^4 CFU/g or lower. These counts indicated acceptable microbiological quality for processed fish products (Lyver, 1997; Yoon *et al.*, 1988). This was expected since the trout products were produced under good manufacturing conditions in the laboratory. The APC of most samples stored at 4°C did not reach 10^7CFU/g (Figures 3.17 and 3.18) by day 28, with the exception of raw wontons. Since the other products were partially or fully cooked prior to packaging, most of the vegetative cells would be killed in the cooking process. Any cell which survived the cooking process should be unable to grow at refrigeration temperature (4°C). In the case of raw wontons, the initial microbial load was high (2×10^5 CFU/g) prior to packaging since they were not cooked. At refrigeration storage conditions, the air and Ageless SS packaged raw wontons only had a shelf-life of 10 days (Figure 3.19). However, the microbial shelf-life of raw wontons was extended for another 9 days for wontons packaged in both 60 and 80% CO_2, indicating that the shelf-life of raw wonton stored at refrigeration temperature could be increased using high levels of CO_2. The results of fried wontons stored at 4°C were not shown since counts were below detectable level, including air packaged samples.

When samples were stored under moderate abuse temperature conditions (12°C), the shelf-life of products was significantly reduced. MAP was unable to inhibit microbial growth in most products. Partially cooked burgers stored at 12°C only had 3-4 days of shelf-life (Figure 3.20), regardless of packaging conditions, while at 4°C their shelf-life could be extended to >28 days (Figure 3.17). All fully cooked burgers had a shelf-life of >28 days, with the exception of air packaged samples stored at 12°C. The longer shelf-life of fully cooked burgers was attributed to the use of MAP technology, as well as their low initial microbial load of $<1.5 \times 10^3$ CFU/g. The shelf-life or air packaged samples was terminated after 8 days. Raw wontons had only a 2-day shelf-life, regardless of packaging conditions, when stored at 12°C. The results of fried wontons were similar to those for fully cooked burgers since most microorganisms present in the products could be killed during the cooking process. All fried wonton samples had a shelf-life of >28 days (counts were not significant enough to be reported) with the exception of air packaged samples which had a shelf-life of 14 days (Figure 3.23).

3.3.2.2. Lactic acid bacteria (LAB) counts

Changes in LAB counts for different packaging treatments of trout burgers and wontons stored at 4 and 12°C are shown in Figures 3.24-3.30. Similar trends in LAB counts were observed as in APC (Section 3.3.2.1.). The LAB counts of partially and fully cooked burgers stored at 4°C remained fairly constant (10^3-10^4 CFU/g) throughout 28 days of storage (Figures 3.24 and 3.25). Raw wontons had higher initial LAB counts (~10^5 CFU/g) than those of fried wontons ($<1.5 \times 10^3$ CFU/g), partially and fully cooked burgers (~10^3 CFU/g). Unlike other products, the LAB counts of raw wontons stored at 4°C increased with storage time. The LAB counts in raw wonton on day 14 were higher in air and Ageless SS than in 60 and 80% CO_2 packaged samples (Figure 3.26), indicating the inhibitory effect of gas packaging on LAB growth. The LAB counts of fried wontons stored at 4°C were too low to be recorded (graph not shown).

Compared to products stored at 4°C, LAB grew fairly well in samples stored at 12°C. The LAB counts for partially cooked burgers stored at 12°C increased to 10^7 CFU/g within a week (Figure 3.27), regardless of packaging conditions. However, LAB counts on fully cooked burgers, at the same storage temperature, remained stable (Figure 3.28) throughout storage. Similar to APC, LAB counts in raw wontons stored at 12°C increased significantly due to their higher initial microbial load (Figure 3.29). LAB counts of Ageless SS and gas packaged fried wontons were too low to be recorded. Air packaged fried wontons stored at 12°C were the only samples of fried wontons to support the growth of LAB (Figure 3.30). This indicated that LAB grew well under air, but their growth in these products was inhibited with the use of MAP.

3.3.2.3. Aerobic and anaerobic spore counts

Counts of aerobic and anaerobic spore forming bacteria were below detectable levels in all raw and cooked products, regardless of packaging conditions and storage temperature (data not shown). This results were surprising since previous studies on similar value-added seafood products (shrimp nuggets) by Lyver (1997) showed significant growth of both aerobic and anaerobic spore forming bacteria, particularly *Bacillus* species.

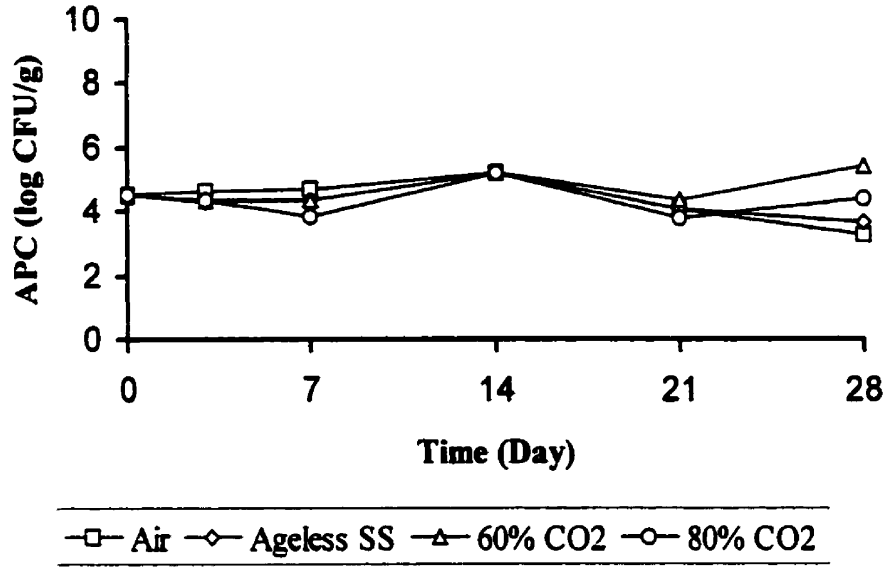

Figure 3.17: Changes in APC of partially cooked burgers packaged under various gas atmospheres and stored at 4°C.

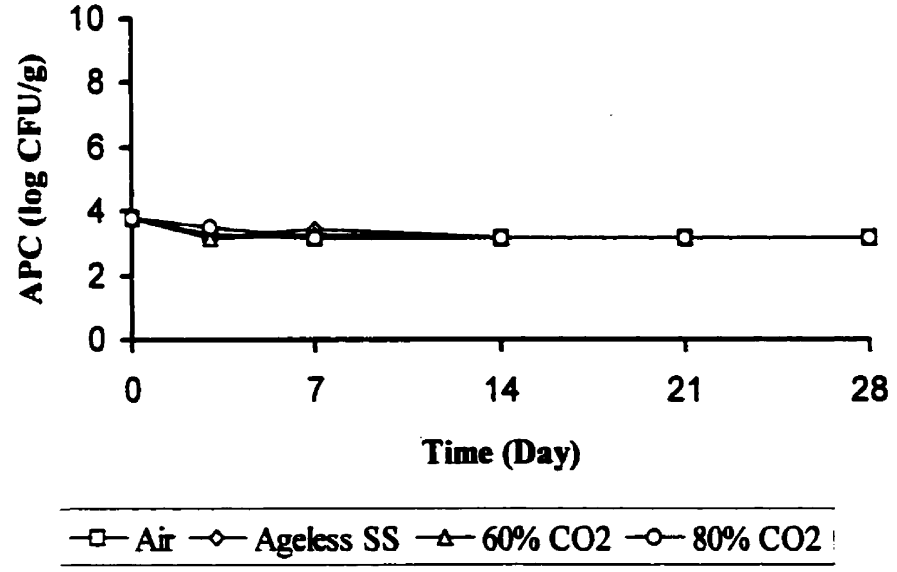

Figure 3.18: Changes in APC of fully cooked burgers packaged under various gas atmospheres and stored at 4°C.

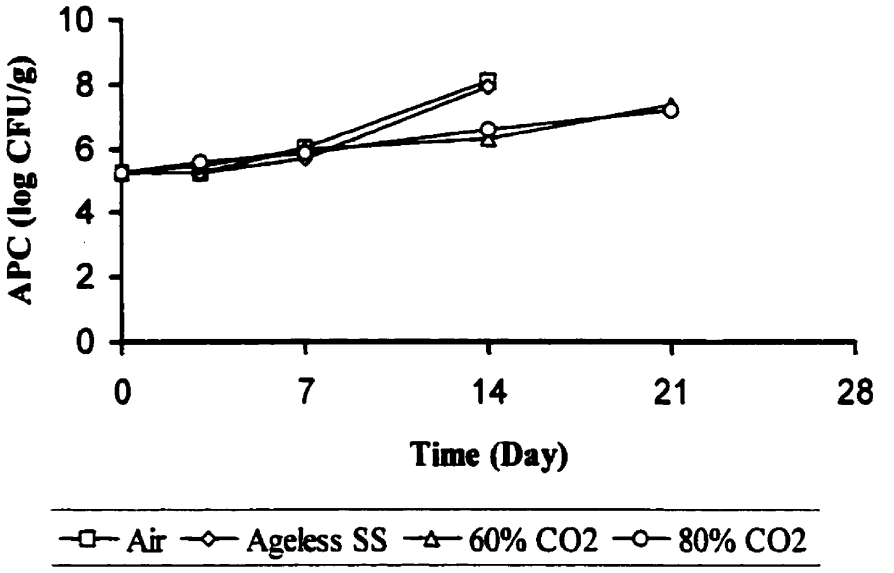

Figure 3.19: Changes in APC of raw wontons packaged under various gas atmospheres and stored at 4°C.

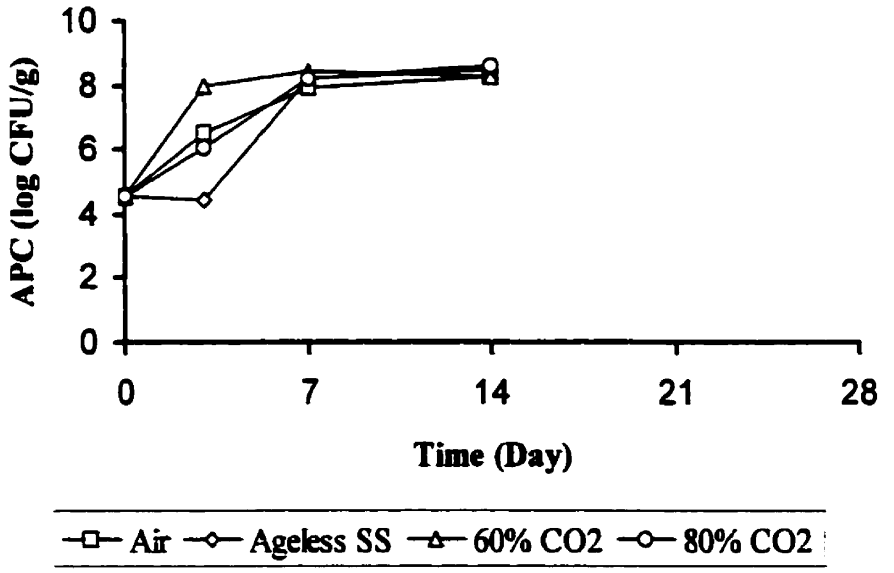

Figure 3.20: Changes in APC of partially cooked burgers packaged under various gas atmospheres and stored at 12°C.

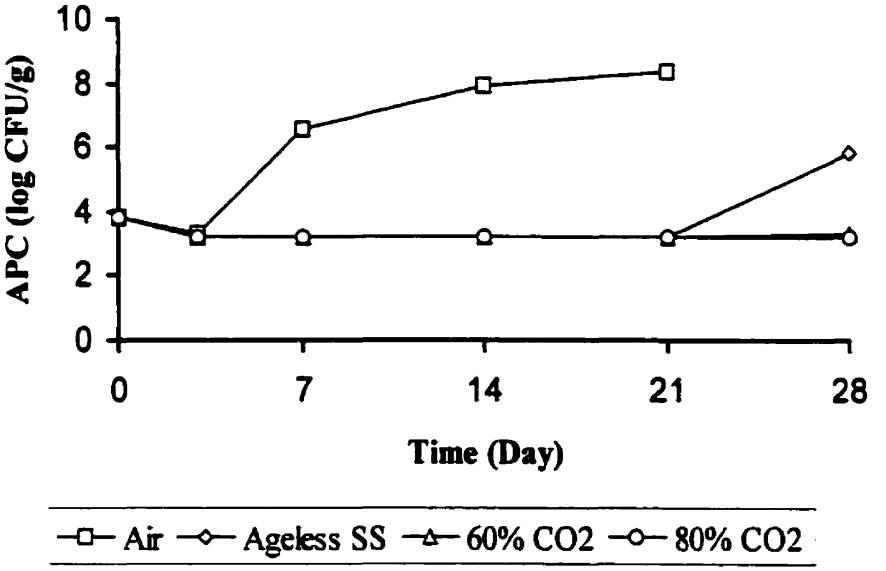

Figure 3.21: Changes in APC of fully cooked burgers packaged under various gas atmospheres and stored at 12°C.

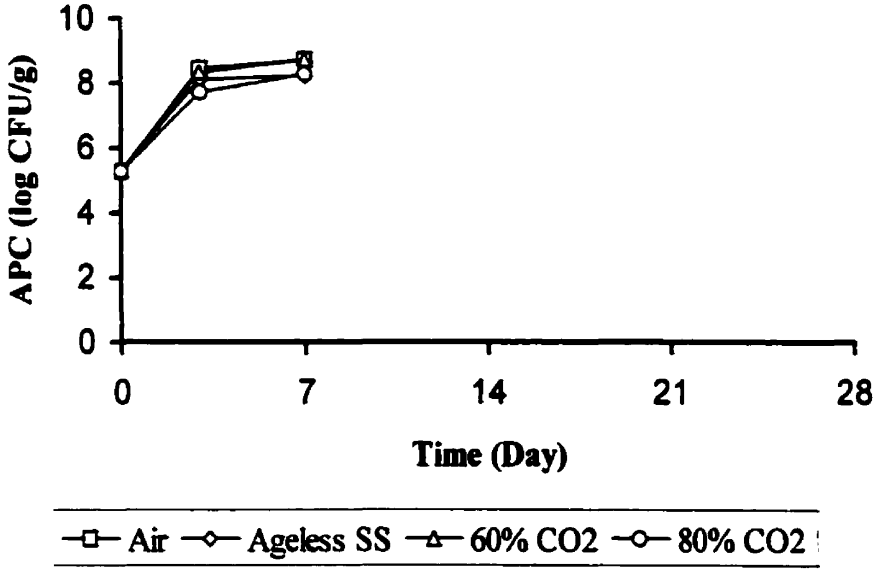

Figure 3.22: Changes in APC of raw wontons packaged under various gas atmospheres and stored at 12°C.

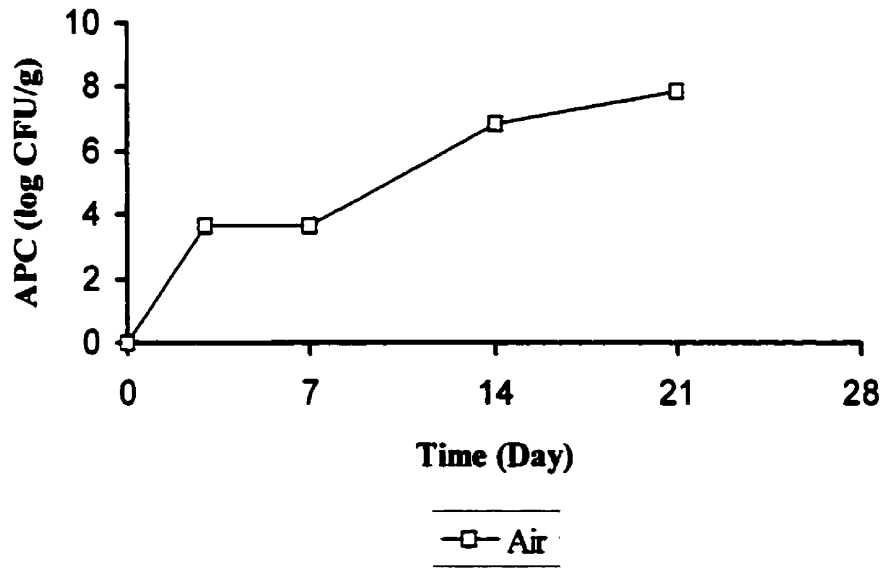

Figure 3.23: Changes in APC of fried wontons packaged under air and stored at 12°C.

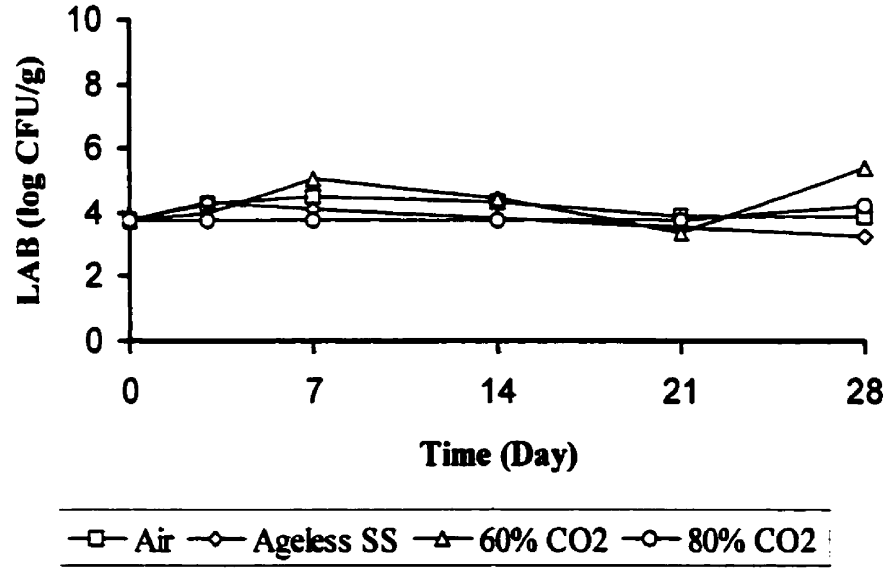

Figure 3.24: Changes in LAB counts of partially cooked burgers packaged under various gas atmospheres and stored at 4°C.

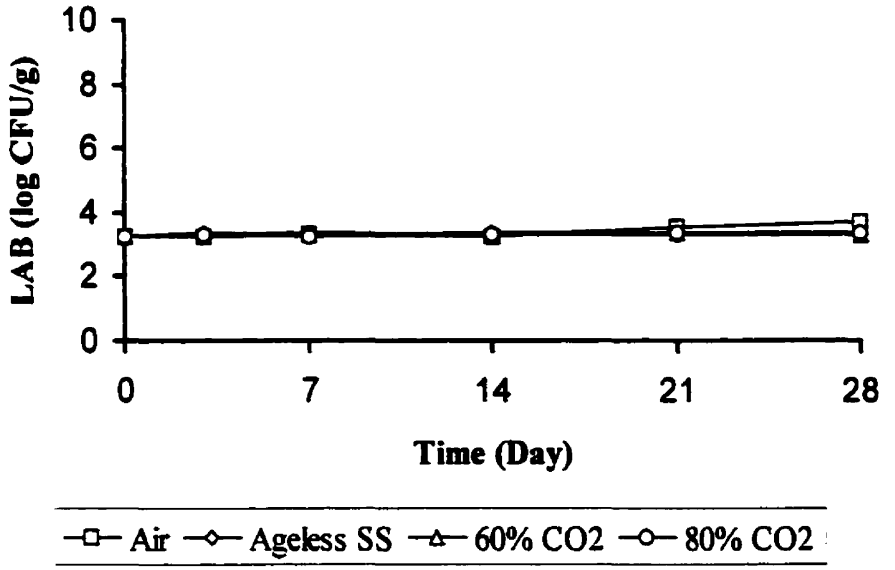

Figure 3.25: Changes in LAB counts of fully cooked burgers packaged under various gas atmospheres and stored at 4°C.

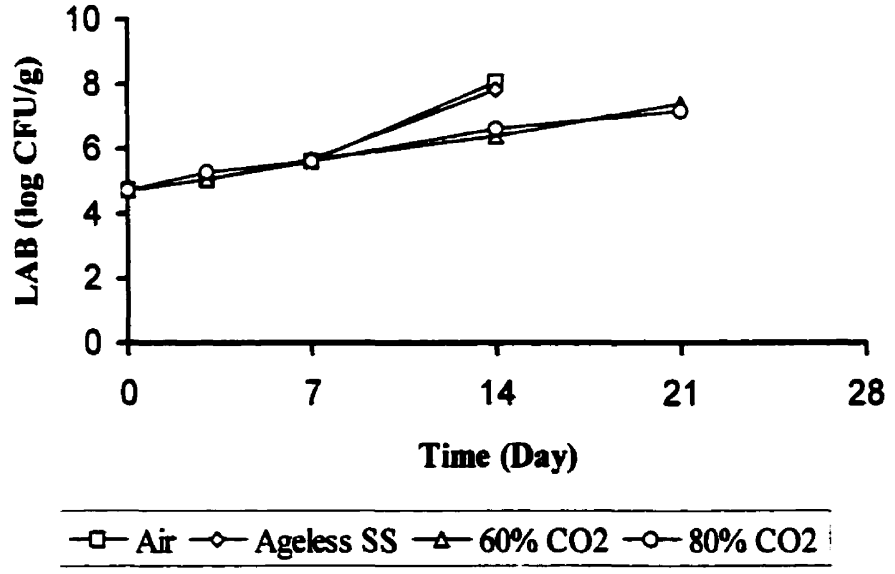

Figure 3.26: Changes in LAB counts of raw wontons packaged under various gas atmospheres and stored at 4°C.

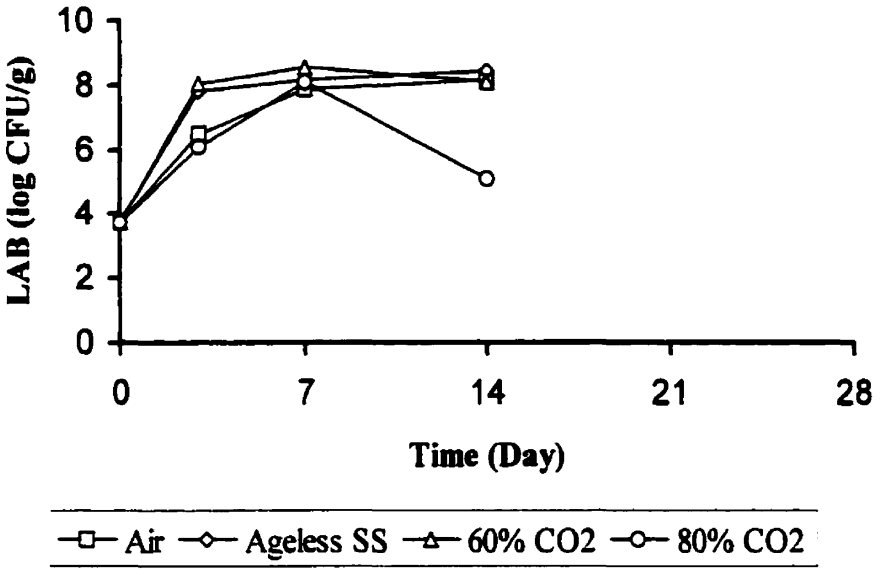

Figure 3.27: Changes in LAB counts of partially cooked burgers packaged under various gas atmospheres and stored at 12°C.

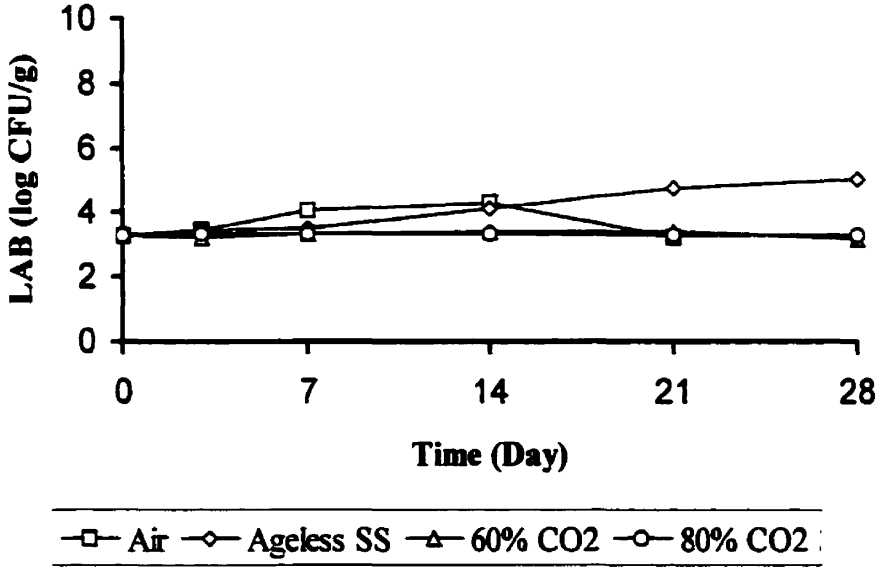

Figure 3.28: Changes in LAB counts of fully cooked burgers packaged under various gas atmospheres and stored at 12°C.

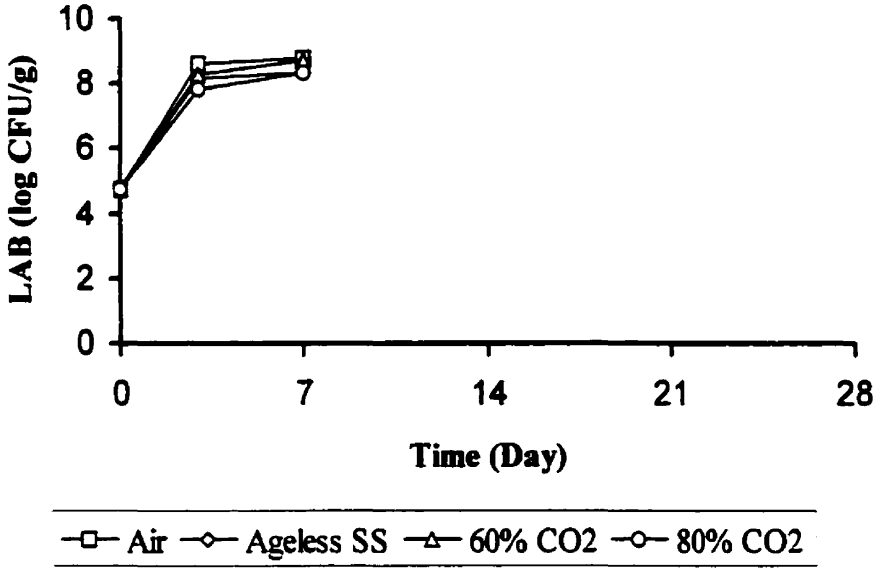

Figure 3.29: Changes in LAB counts of raw wontons packaged under various gas atmospheres and stored at 12°C.

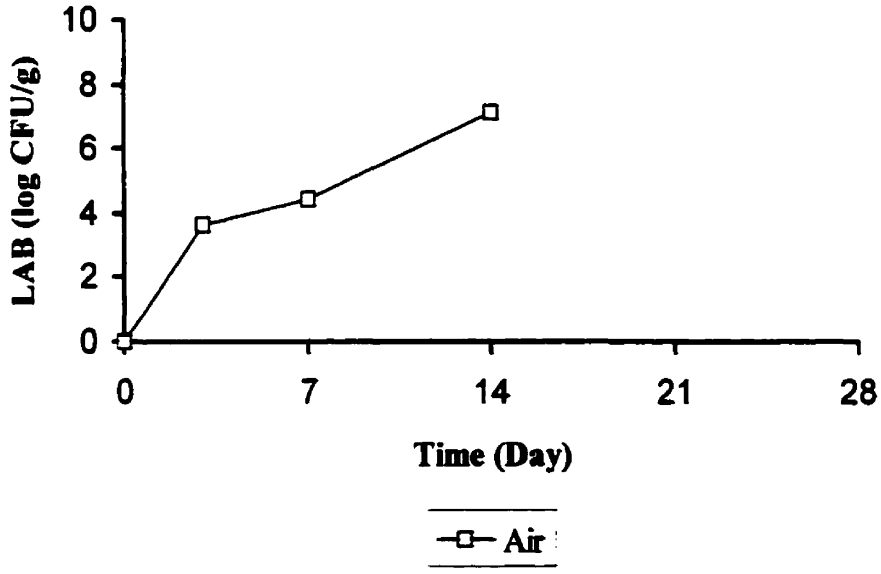

Figure 3.30: Changes in LAB counts of fried wontons packaged under air and stored at 12°C.

3.3.3. Changes in pH

The changes in pH values of trout products packaged under different atmospheres and stored at 4 and 12°C are shown in Figures 3.31-3.38 respectively. Similar trends were observed for partially, fully cooked trout burgers and fried wontons stored at 4°C, i.e., their pH values remained fairly constant throughout storage (Figures 3.31, 3.32 and 3.34). There was a slight decrease of pH values in gas packaged samples may be explained by the dissolution of CO_2 from the package headspace. In the case of raw wontons stored at 4°C, pH values decreased from ~6.5 to ~5 after 14 days of storage (Figure 3.33). This was attributed to acid production by LAB in raw trout wontons. The slight delay of the decrease in pH values in gas packaged raw wontons may be attributed to the inhibition of the growth of LAB in these samples by MAP.

A slight decrease in pH was observed for partially cooked burgers stored at 12°C (Figure 3.35), while the pH of raw wontons at the same temperature decreased to ~ 4.5 in just 3 days (Figure 3.37) due to the growth of LAB. The pH values did not change significantly in both fully cooked burgers and fried wontons stored under similar conditions (Figures 3.36 and 3.38). The results observed for value-added trout products (both raw and cooked) were consistent of those observed in raw and cooked value-added shrimp products by Lyver (1997). An increase in product pH during storage may be explained by the buffering effect of muscle proteins and release of amino acids, probably as a result of proteolytic activity of facultatively anaerobic spoilage bacteria (Dufresne, 1999).

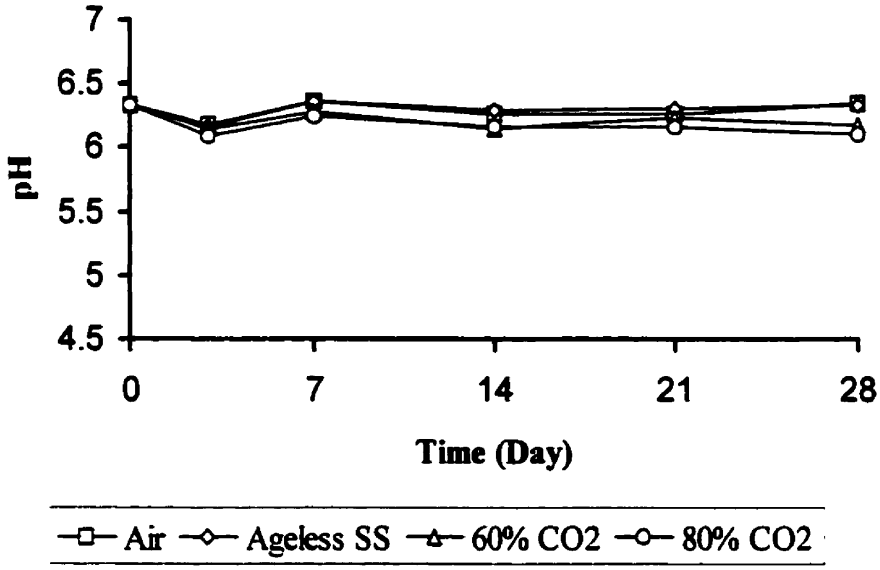

Figure 3.31: Changes in pH values of partially cooked burgers packaged under various gas atmospheres and stored at 4°C.

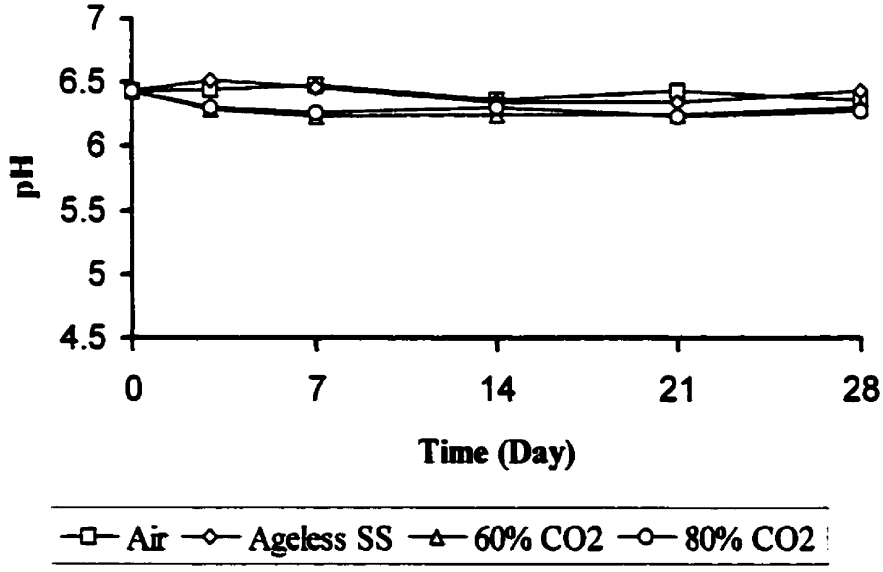

Figure 3.32: Changes in pH values of fully cooked burgers packaged under various gas atmospheres and stored at 4°C.

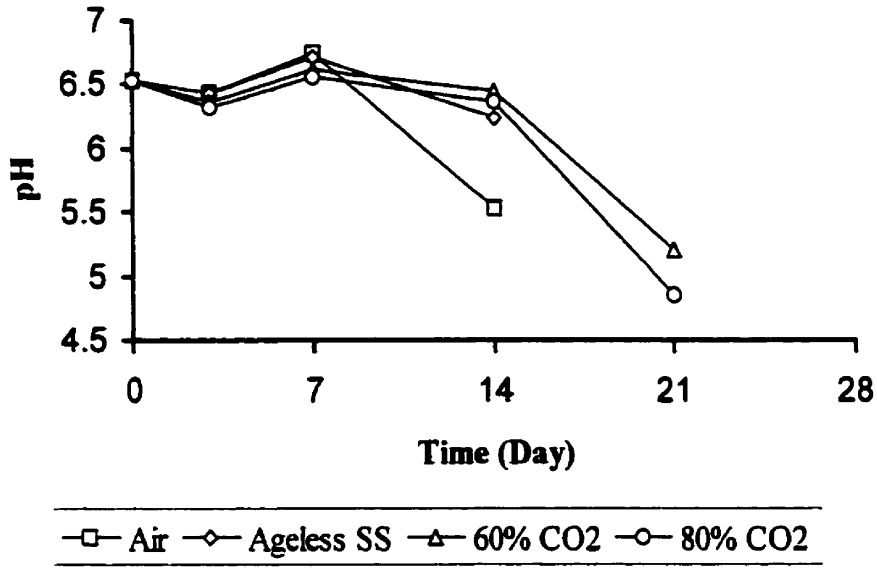

Figure 3.33: Changes in pH values of raw wontons packaged under various gas atmospheres and stored at 4°C.

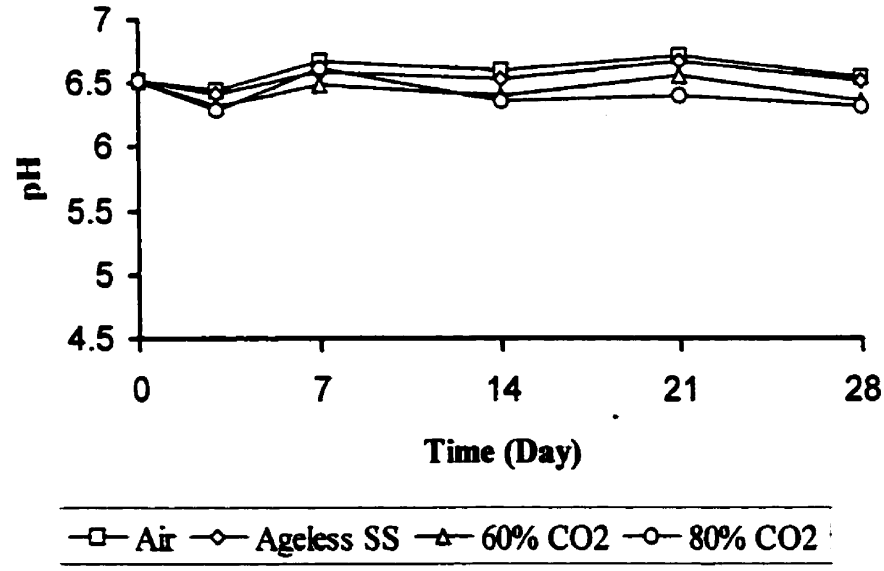

Figure 3.34: Changes in pH values of fried wontons packaged under various gas atmospheres and stored at 4°C.

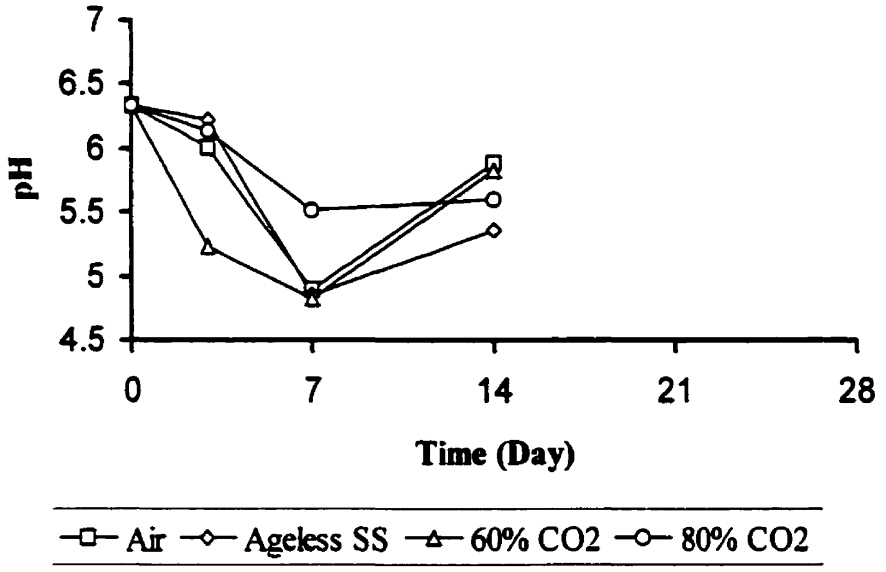

Figure 3.35: Changes in pH values of partially cooked burgers packaged under various gas atmospheres and stored at 12°C.

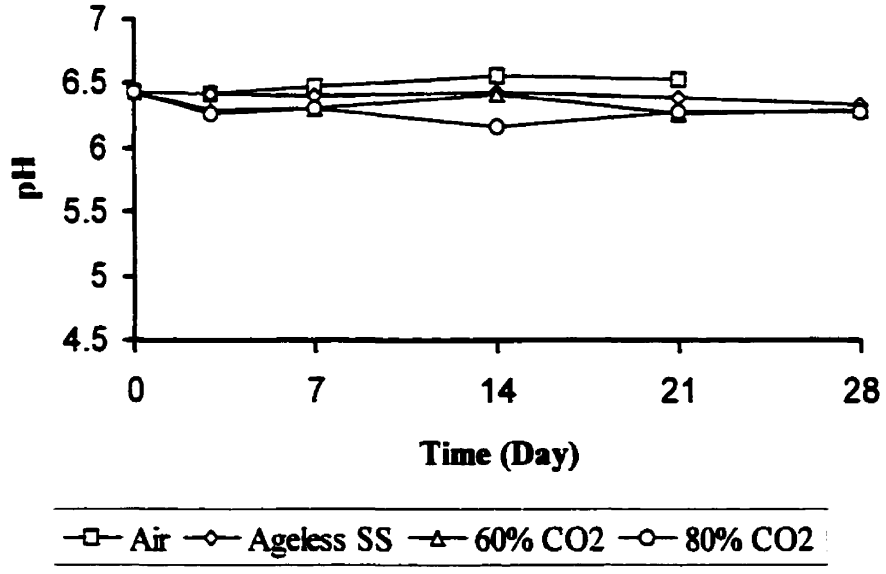

Figure 3.36: Changes in pH values of fully cooked burgers packaged under various gas atmospheres and stored at 12°C.

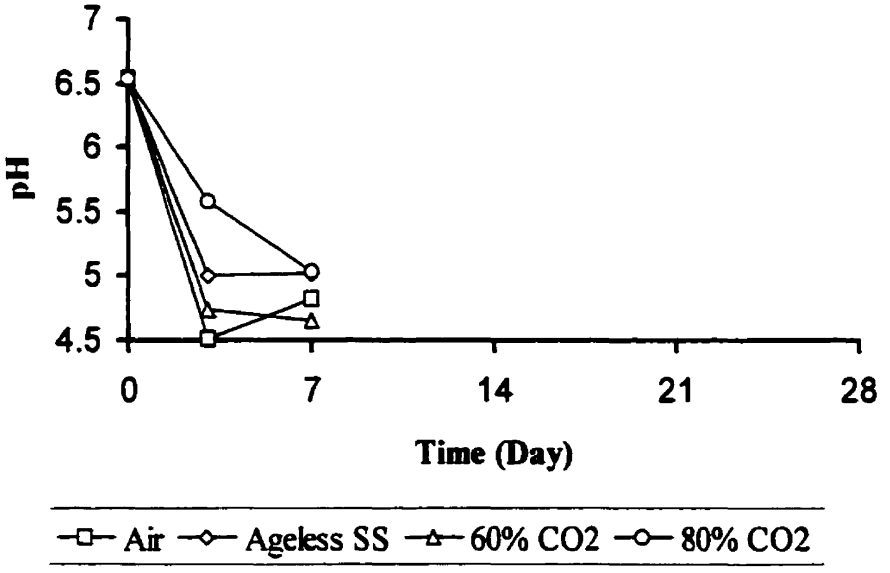

Figure 3.37: Changes in pH values of raw wontons packaged under various gas atmospheres and stored at 12°C.

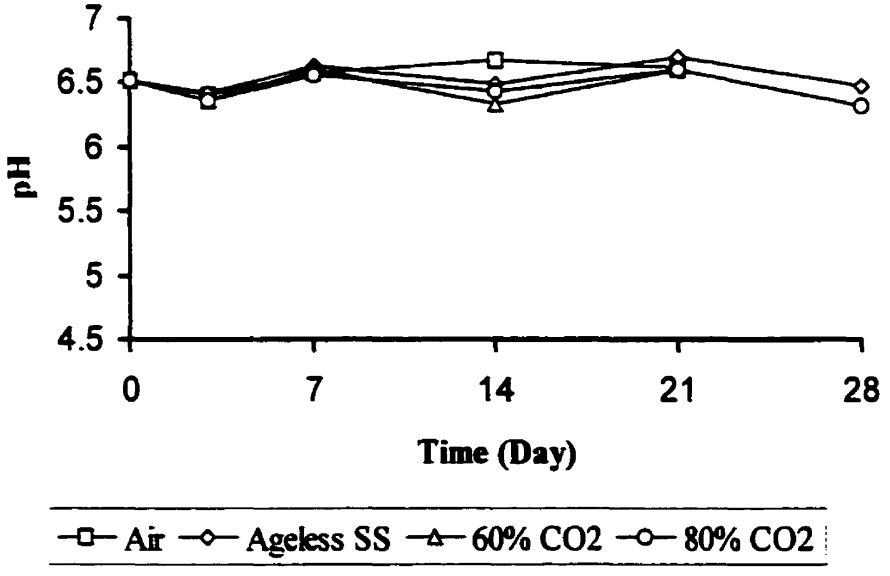

Figure 3.38: Changes in pH values of fried wontons packaged under various gas atmospheres and stored at 12°C.

3.3.4. Sensory Evaluation

Sensory evaluation scores of odor, color, texture and overall acceptability for trout burgers and wontons stored under different atmospheres are summarized in Tables 3.3-3.5. No sensory evaluation was performed on raw wontons since the purpose of including raw wontons in the study was to examine the physical, chemical and microbiological differences between uncooked and cooked value-added trout products during storage. Products were regarded as unacceptable when a score of 3.5 on a subjective scale of 7 was reached (Greer, 1993).

Sensory scores of all partially cooked burgers were fairly constant (Table 3.3), with the exception of air packaged samples stored at 12°C. Air packaged samples stored at 12°C had the lowest score compared to samples other partially cooked burgers stored at the same temperature. Shelf-life of these air packaged samples was terminated after 12 days of storage. Products developed sharp, acidic and musty odors when their shelf-life was terminated. This may be attributed to the growth of LAB and other fermentative bacteria, which have been reported to cause off-odors by depleting glucose, enhancing amino acid breakdown and producing putrefactive odors in products (Newton and Rigg, 1979). However, the color, texture and overall acceptability scores of air packaged, partially cooked burgers stored at 12°C were still acceptable by the time their shelf-life was terminated.

Similar trends were observed for fully cooked burgers and fried wontons (Tables 3.4 and 3.5), with air packaged samples stored at 12°C having lower scores compared to others. However, the decrease in sensory scores was not as significant as those in partially cooked burgers. No sensory score (regardless of different sensory attributes) of fully cooked burgers and fried wontons decreased to ≤ 3.5 after 28 days. There was no conclusive relationship between gas packaging conditions and storage temperature in sensory scores.

Table 3.3: Results of sensory evaluation of partially cooked burgers packaged under various gas atmospheres and stored at 4 and 12°C.

Packaging treatment	Sensory score[1]							
	Odor		Color		Texture		Overall acceptability	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sensory score @ 4°C[2]								
Air		5.0		5.6		4.6		4.4
Air + Ageless SS	6.5	5.5	6.1	5.4	5.7	5.3	6.2	5.4
60% CO_2		5.4		5.0		5.4		5.3
80% CO_2		5.4		5.7		5.5		5.5
Sensory score @ 12°C[3]								
Air		3.1		4.7		4.3		3.5
Air + Ageless SS	6.5	5.1	6.1	5.3	5.7	4.4	6.2	5.3
60% CO_2		5.8		5.9		5.0		5.7
80% CO_2		5.2		5.3		4.8		4.8

[1] Based on a 7-point hedonic scale (7 = Extremely desirable, 1 = Extremely undesirable)

[2] Final sensory scores obtained on day 28

[3] Final sensory scores obtained on day 14

Table 3.4: Results of sensory evaluation of fully cooked burgers packaged under various gas atmospheres and stored at 4 and 12°C.

Packaging treatment	Sensory Score[1]							
	Odor		Color		Texture		Overall acceptability	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sensory score @ 4°C								
Air		4.9		5.7		4.5		4.8
Air + Ageless SS	6.6	5.3	6.3	5.6	6.1	4.7	6.4	5.0
60% CO_2		5.9		5.9		4.8		5.7
80% CO_2		6.0		5.8		4.7		5.6
Sensory score @ 12°C								
Air[2]		3.7		5.6		5.4		4.3
Air + Ageless SS	6.6	5.4	6.3	5.5	6.1	4.6	6.4	5.1
60% CO_2		5.8		5.7		5.1		5.4
80% CO_2		4.8		5.4		5.0		4.8

[1] Based on a 7-point hedonic scale (7 = Extremely desirable, 1 = Extremely undesirable)

[2] Final sensory scores obtained on day 21

Table 3.5: Results of sensory evaluation of fried wontons packaged under various gas atmospheres and stored at 4 and 12°C.

Packaging treatment	Sensory Score[1]							
	Odor		Color		Texture		Overall acceptability	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sensory score @ 4°C								
Air		4.9		5.3		4.3		4.7
Air + Ageless SS	6.3	4.3	6.3	3.0	6.4	3.1	6.3	3.3
60% CO_2		5.2		6.0		3.2		4.3
80% CO_2		5.4		5.4		4.5		5.3
Sensory score @ 12°C								
Air[2]		4.1		4.0		3.8		3.8
Air + Ageless SS	6.3	4.9	6.3	4.6	6.4	3.6	6.3	4.4
60% CO_2		4.7		4.5		3.8		4.0
80% CO_2		6.2		5.7		4.5		5.7

[1] Based on a 7-point hedonic scale (7 = Extremely desirable, 1 = Extremely undesirable)

[2] Final sensory scores obtained on day 21

3.4. Shelf-Life of Value-Added Trout Burgers and Trout Wontons

Estimations of microbial and sensory shelf-life for value-added trout burgers and wontons packaged under different gas atmospheres and stored at 4 and 12°C are summarized in Tables 3.6-3.9. The microbial shelf-life of products was based on the time necessary for the APC to be $>10^7$ CFU/g. The shortest time to reach a score of 3.5 (rejection point in acceptability scale) from either odor, color, texture or overall acceptability was used as an indicator of sensory shelf-life.

Partially cooked burgers stored at 4°C had a microbial shelf-life of >28 days (Table 3.6). However, if the products were stored under temperature abuse conditions, shelf-life was reduced to 3-4 days. Regarding the sensory shelf-life, most products at 12°C were sensorially acceptable although their microbial shelf-life was terminated. Air packaged samples stored at 12°C had the lowest sensorial scores and were rejected after 12 days.

Fully cooked trout burgers had a microbial and sensorial shelf-life of >28 days at both 4 and 12°C (Table 3.7), with the exception of air packaged samples which had a microbial shelf-life of only 8 days at 12°C. However, their sensory shelf-life was >28 days, which may due to the masking of off-odor by the stronger, desirable odor associated with fried food products.

MAP had a significant effect in extending the shelf-life of raw wontons. However, the effectiveness of MAP was dependant on storage temperature. Raw wontons stored under moderate temperature abuse conditions had a shelf-life of 2 days (Table 3.8). However, if stored under refrigeration temperature, the shelf-life of raw wontons could be extended for 8-17 days, depending on packaging treatment. The addition of Ageless SS to product package did not significantly increase shelf-life of the products. However, gas packaging, using elevated levels of CO_2, could extend shelf-life of raw wontons for >1 week.

Similar to fully cooked trout burgers, fried wontons could be stored for >28 days (Table 3.9). Even under moderate temperature abuse conditions, fried wontons had a microbial shelf-life of >28 days, again with the exception of air packaged samples which had only 2 weeks of shelf-life at 12°C. Fried wontons had a sensory shelf-life of >28 days, regardless of packaging condition and storage temperature.

When products were cooked, a longer microbial shelf-life could be achieved, irrespective of packaging treatment and storage temperature. This can be attributed to the destruction of heat sensitive microorganisms in the cooking process. Therefore, shelf-life of products could be enhanced with less initial microbial load prior to packaging, and the effective use of MAP under temperature control.

Table 3.6: Estimated microbial and sensory shelf-life of partially cooked trout burgers packaged under various gas atmospheres and stored at 4 and 12°C.

Temperature (°C)	Packaging condition	Microbial shelf-life[1] (day)	Sensory shelf-life[2] (day)
4	Air	28+	28+
	Air + Ageless SS	28+	28+
	60% CO_2	28+	28+
	80% CO_2	28+	28+
12	Air	4	12
	Air + Ageless SS	3	28+
	60% CO_2	3	28+
	80% CO_2	4	28+

[1] Rejection point: time (days) to reach APC of 10^7 CFU/g

[2] Rejection point: shortest time (days) to reach a score of ≤ 3.5 from either odor, color, texture or overall acceptability

Table 3.7: Estimated microbial and sensory shelf-life of fully cooked trout burgers packaged under various gas atmospheres and stored at 4 and 12°C.

Temperature (°C)	Packaging condition	Microbial shelf-life[1] (day)	Sensory shelf-life[2] (day)
4	Air	28+	28+
	Air + Ageless SS	28+	28+
	60% CO_2	28+	28+
	80% CO_2	28+	28+
12	Air	8	28+
	Air + Ageless SS	28+	28+
	60% CO_2	28+	28+
	80% CO_2	28+	28+

[1] Rejection point: time (days) to reach APC of 10^7 CFU/g

[2] Rejection point: shortest time (days) to reach a score of ≤ 3.5 from either odor, color, texture or overall acceptability

Table 3.8: Estimated microbial and sensory shelf-life of raw wontons packaged under various gas atmospheres and stored at 4 and 12°C.

Temperature (°C)	Packaging condition	Microbial shelf-life[1] (day)
4	Air	10
	Air + Ageless SS	11
	60% CO_2	19
	80% CO_2	19
12	Air	2
	Air + Ageless SS	2
	60% CO_2	2
	80% CO_2	2

[1] Rejection point: time (days) to reach APC of 10^7 CFU/g

Table 3.9: Estimated microbial and sensory shelf-life of fried wontons packaged under various gas atmospheres and stored at 4 and 12°C.

Temperature (°C)	Packaging condition	Microbial shelf-life[1] (day)	Sensory shelf-life[2] (day)
4	Air	28+	28+
	Air + Ageless SS	28+	28+
	60% CO_2	28+	28+
	80% CO_2	28+	28+
12	Air	14	28+
	Air + Ageless SS	28+	28+
	60% CO_2	28+	28+
	80% CO_2	28+	28+

[1] Rejection point: time (days) to reach APC of 10^7 CFU/g

[2] Rejection point: shortest time (days) to reach a score of ≤ 3.5 from either odor, color, texture or overall acceptability

3.5. Conclusions

This study has shown that the use of MAP technology could extend both the microbial and sensory shelf-life of formulated value-added trout products (trout burgers and trout wontons). A longer shelf-life is possible by cooking the product prior to packaging. However, strict temperature control is necessary to ensure the effectiveness of MAP as temperature has a synergistic effect with MAP in delaying spoilage of products.

Most fully cooked products (burgers and wontons) had a shelf-life of >28 days. Partially cooked burgers and raw wontons had relatively a shorter shelf-life (2-4 days at 12°C) compared to the fully cooked products, but their shelf-life could be extended using MAP and strict temperature control. These results are in agreement with previous studies using MAP to extend the shelf-life of value-added seafood products (Lyver, 1997).

CHAPTER 4:
CHALLENGE STUDIES WITH *LISTERIA MONOCYTOGENES*

4.1. Introduction

MAP has been shown to extend the shelf-life of value-added seafood products by inhibiting the growth of aerobic spoilage bacteria (Chapter 3). However, concern has been expressed about the public health safety of MAP seafood with respect to the potential growth of facultative and/or anaerobic psychrotrophic pathogenic bacteria under low O_2 concentrations, such as *Listeria monocytogenes*. The incidence of *L. monocytogenes* contamination in imported and domestic seafood in the United States has been reported to be between 5 and 6% (McCarthy, 1997). Recently, it was isolated from both whole fish and fillets of aquacultured rainbow trout (McAdams, 1996).

L. monocytogenes has been found to grow at refrigeration temperatures under various modified atmospheres. The effect of MAP on the growth of *L. monocytogenes* varies according to the atmosphere used and the food product itself. It was shown that a modified atmosphere consisting of 100% CO_2 was more inhibitory to the growth of a four-strain mixture of *Listeria* spp., including *L. monocytogenes* on blood agar plates, than any other atmosphere (Razavilar and Genigeorgis, 1992). In addition, the growth of *Listeria* spp. was delayed in greater extent at lower temperature. Other authors have shown similar results, including Gill and Reichel (1989).

As a result of these observations, it was necessary to investigate the potential growth of *L. monocytogenes* in the formulated value-added rainbow trout products stored under MAP at refrigeration and temperature abuse conditions. The objectives of this study were to monitor the physical, chemical and microbiological changes in product samples inoculated with *L. monocytogenes*, packaged under various atmospheres and stored at refrigeration (4°C) and moderate temperature abuse conditions (12°C).

4.2. Materials and Methods

4.2.1. Preparation of Samples

Value-added trout burgers and wontons were prepared as outlined in Sections 3.2.1. and 3.2.2. respectively.

4.2.2. Preparation of Bacterial Strains and Inoculation

Five strains of *L. monocytogenes* were obtained from the culture collection of Dr. J. Farber (Bureau of Microbial Hazards, Health Protection Branch, Health Canada, Ottawa, Ontario, Canada). The strains used included Scott A (human isolate), and Health Protection Branch's (HPB's) strains 323 (shrimp isolate), 392 (lobster isolate), 439 (crab isolate) and 976 (salmon isolate). All cultures were maintained on TSA (Difco Laboratories) plates and transferred monthly to ensure viability. The inoculum was prepared by transferring isolated colonies of each strain from TSA plates into separate test tubes containing 5 ml of Tryptic Soy Broth with 0.6% Yeast Extract (TSBYE, Difco Laboratories) and incubated for 15-18 hours at 35°C to give a suspension of ~10^9 CFU/ml. Two hundred µl of each suspension was added to 9 ml 0.1% sterile peptone water (Difco laboratories) to give a five strains *L. monocytogenes* mixture of ~10^8 CFU/ml. One ml of this suspension was placed into another 9 ml of 0.1% sterile peptone water to give a 1:10 dilution. Subsequent dilutions were carried out until the concentration of the suspension reached ~10^5 CFU/ml. Samples (~100 g/bag) were inoculated by injecting 100 µl of this suspension into the filling of products to give a final inoculum level of ~10^2 CFU/g. Control samples were inoculated in a similar manner with the same volume of 0.1% sterile peptone water. The inoculation was carried out in a Purifier™ Class II Safety Cabinet (Labconco, Model 36205-04, Labconco, Kansas, Missouri, United States) equipped with a HEPA filter to ensure minimal contamination of the samples and the surrounding environment as well as the safety of the research personnel.

4.2.3. Packaging of Samples

Partially/fully cooked trout burgers (1 per bag) and raw/fried wontons (2 per bag) were packaged in 210 mm x 210 mm high gas barrier bags (OTR: 11.6 cc/m^2/day at 24°C, 0% relative humidity (RH); Cryovac Sealed Air Corporation). Samples were packaged under the following conditions: air, air with an Ageless SS-200 oxygen absorbent (Mitsubishi Gas Chemical American Inc.), 60% and 80% CO_2 (balance N_2). Air packaged samples were sealed using an Impulse Heat Sealer. Ageless SS-200 oxygen absorbents were inserted into the appropriate bags which were then sealed as described above. Gas packaging was done using a chamber type, heat seal packaging machine (Model A300/42, Multivac Inc.). A Smith's proportional gas mixer (Model 299-028, Tescom Corporation) was used to give the desired proportions of O_2, CO_2 and N_2 in the package headspace. Duplicate samples of each packaging conditions were stored at 4 and 12°C and were monitored for physical, chemical and microbiological changes throughout the study (28 days storage trial) or until shelf-life was terminated. Most samples were tested on day 0, 3, 7, 14, 21 and 28 with the exception of raw wontons stored at 12°C which were tested on day 0, 1, 2, 3, 4 and 7 due to their shorter shelf-life.

4.2.4. Headspace Gas Analysis

Headspace gas analysis was carried out as described in Section 3.2.4.

4.2.5. Microbiological Analysis

Samples were evaluated for APC, LAB, *L. monocytogenes*, aerobic and anaerobic spores count at each sampling day. All samples were aseptically transferred into a sterile stomacher bag and 0.1% sterile peptone water added to achieve a 1:3 dilution. The bag was

stomached for 1 min in a Stomacher 400 LabBlender (A.J. Seward). One ml of this suspension was aseptically transferred into 9 ml of 0.1% sterile peptone water to give a 1:30 dilution. Subsequent dilutions were carried out as required to give countable plates. APC, aerobic and anaerobic spore counts were determined by placing appropriate dilutions on TSA which LAB counts were enumerated using MRS agar (Difco Laboratories). *L. monocytogenes* counts were enumerated using PALCAM agar (Oxoid, Unipath Inc., Basingstoke, Hampshire, United Kingdom) supplemented with Palcam Selective Supplement (SR150E). For all counts, 0.1 ml of the appropriate dilution was plated in duplicate using a spread plate technique. Plates were incubated aerobically with the exception of LAB and anaerobic spore counts which were incubated anaerobically using anaerobic Jars (BBL Microbiology Systems). All plates were incubated at 35°C for 48 hours. Spores, both aerobic and anaerobic, were determined by first heat shocking the appropriate dilutions at 75°C for 20 min. Countable plates (30-300 colonies) were reported as $\log_{10}$ Colony Forming Unit per gram of sample (log CFU/g). Control samples were checked for the presence of *L. monocytogenes* on the first and the day when shelf-life of products was terminated, using the method described previously.

4.2.6. pH Measurement

The pH of each homogenized samples was measured using a previously calibrated (buffer solutions of pH 4 and 7, Fisher Scientific) Corning pH meter (Model 220, Corning Glass Works) with a gel filled polymer body combination electrode with Ag/AgCl reference (Model 13-620-104, Fisher Scientific). The electrode was inserted directly into the suspension of stomached samples (1:3 dilution) after microbiological testing procedures were completed. Analysis (in duplicate) was carried out at each sampling time.

4.3. Results and Discussions

4.3.1. Headspace Gas Analysis

Changes in headspace gas composition (O_2 and CO_2) of inoculated partially and fully cooked trout burgers, and raw and fried wontons stored at 4 and 12°C are shown in Tables 4.1-4.4. Similar changes in headspace gas composition of both control and inoculated products, i.e., a decrease in O_2 and an increase in CO_2, were observed as in shelf-life studies (Chapter 3, Section 3.3.1.). The decrease in headspace O_2 and the increase in headspace CO_2 were the result of microbial activity. There was slight decrease in headspace CO_2 in gas packaged samples due to the dissolution of gaseous CO_2 into the aqueous phase of food products. The various final sampling days for analysis were attributed to differences in shelf-life of these value-added trout products.

For inoculated partially cooked burgers stored at both 4 and 12°C (Table 4.1), the level of headspace O_2 decreased gradually with time for most samples with the exception of Ageless SS packaged samples which decreased to <1% within 3 days. The O_2 level also remained low (<1%) for gas packaged samples throughout storage. Headspace CO_2 of most partially cooked burgers remained fairly constant with the exception of air packaged samples stored at 4°C. The increase in headspace CO_2 (~10%) in these samples after 28 days may be attributed to the growth of *L. monocytogenes* since no significant increase (~3%) was observed in control samples (data not shown).

The levels of O_2 and CO_2 of inoculated fully cooked burgers and fried wontons remained fairly constant throughout storage (Table 4.2 and 4.4), with the exception of air packaged fully cooked trout burgers stored at 12°C. Headspace CO_2 of these samples increased significantly (~14%) when their shelf-life was terminated. This is partly due to the growth of *L. monocytogenes* in these products since the increase in headspace CO_2 of control samples was only ~3% (data not shown) for the same storage period.

Changes in headspace gas of inoculated raw wontons are summarized in Table 4.3. Similar to other products, results of raw wontons showed a gradual decrease in headspace O_2 and increase in headspace CO_2. No significant difference was observed between inoculated and control samples.

Table 4.1: Changes in headspace O_2 and CO_2 of inoculated partially cooked burgers packaged under various gas atmospheres and stored at 4 and 12°C.

Temperature (°C)	Packaging condition	Headspace gas level (%v/v)			
		O_2		CO_2	
		Initial	Final	Initial	Final
4[1]	Air	21.0	3.4	0	9.9
	Air + Ageless SS	21.0	0	0	0.3
	60% CO_2	1.9	0	61.9	48.5
	80% CO_2	2.0	0.4	81.1	70.3
12[2]	Air	21.0	16.9	0	3.0
	Air + Ageless SS	21.0	0	0	0.3
	60% CO_2	1.9	0	61.9	53.6
	80% CO_2	2.0	0.6	81.1	74.2

[1] Final results were obtained on day 28

[2] Final results were obtained on day 7

Table 4.2: Changes in headspace O_2 and CO_2 of inoculated fully cooked burgers packaged under various gas atmospheres and stored at 4 and 12°C.

Temperature (°C)	Packaging condition	Headspace gas level (%v/v)[1]			
		O_2		CO_2	
		Initial	Final	Initial	Final
4	Air	21.0	16.9	0	1.4
	Air + Ageless SS	21.0	0	0	0.2
	60% CO_2	1.4	0	61.9	47.3
	80% CO_2	3.3	0.6	82.0	68.8
12	Air[2]	21.0	7.4	0	14.4
	Air + Ageless SS	21.0	0	0	0.6
	60% CO_2	1.4	0	61.9	49.3
	80% CO_2	3.3	0	82.0	71.2

[1] Final results were obtained on day 28

[2] Final results were obtained on day 14

Table 4.3: Changes in headspace O_2 and CO_2 of inoculated raw wontons packaged under various gas atmospheres and stored at 4 and 12°C.

Temperature (°C)	Packaging condition	Headspace gas level (%v/v)			
		O_2		CO_2	
		Initial	Final	Initial	Final
4[1]	Air	21.0	18.9	0	3.6
	Air + Ageless SS	21.0	1.7	0	0.3
	60% CO_2	0	1.1	61.4	56.4
	80% CO_2	0	0.5	80.3	71.9
12[2]	Air	21.0	18.3	0	4.8
	Air + Ageless SS	21.0	1.1	0	1.1
	60% CO_2	0	0.9	61.4	55.7
	80% CO_2	0	0.5	80.3	73.1

[1] Final results were obtained on day 7

[2] Final results were obtained on day 4

Table 4.4: Changes in headspace O_2 and CO_2 of inoculated fried wontons packaged under various gas atmospheres and stored at 4 and 12°C.

Temperature (°C)	Packaging condition	Headspace gas level (%v/v)[1]			
		O_2		CO_2	
		Initial	Final	Initial	Final
4	Air	21.0	19.7	0	0.6
	Air + Ageless SS	21.0	0	0	0.1
	60% CO_2	0	0	61.4	56.2
	80% CO_2	0	0	80.3	75.0
12	Air[2]	21.0	18.5	0	3.0
	Air + Ageless SS	21.0	0	0	0.2
	60% CO_2	0	0.3	61.4	51.1
	80% CO_2	0	0	80.3	70.5

[1] Final results were obtained on day 28

[2] Final results were obtained on day 14

4.3.2. Microbiological Analysis

Changes in *L. monocytogenes* counts for inoculated trout burgers and wontons stored under various gas atmospheres and stored at 4 and 12°C are shown in Figures 4.1-4.7. *L. monocytogenes* was not detected in any control samples stored at both 4 and 12°C (data not shown).

Gas packaging in 80% CO_2 was found to effectively inhibit the growth of *L. monocytogenes* in partially and fully cooked trout burgers, and fried trout wontons (Figures 4.1- 4.3). Similar results were reported by Sheridan *et al.* (1995) and Avery *et al.* (1994), who reported inhibition of *L. monocytogenes* in food products packaged under elevated CO_2 levels. For partially cooked burgers stored at 4°C (Figure 4.1), *L. monocytogenes* counts increased from an initial level of ~10^2 CFU/g to ~10^4 CFU/g after 28 days, with the exception of 80% CO_2 packaged samples. Similar trends were observed in both inoculated fully cooked burgers and fried wontons stored at 4°C (Figures 4.2 and 4.3). However, the growth of *L. monocytogenes* in these products was more rapid and more abundant. This may be due to less competition in these products compared to partially cooked trout burgers which had higher initial microbial loads. For both fully cooked burgers and fried wontons stored at 4°C, higher *L. monocytogenes* counts (>10^7 CFU/g) were observed in air packaged samples compared to products packaged under other gas atmospheres. Counts of *L. monocytogenes* were reduced >1 log by MAP, including both gas packaging and the use of oxygen absorbent. Furthermore, a delaying effect for the growth of *L. monocytogenes* (longer lag phase) was observed in gas packaged products. These results confirm the delaying effects of elevated levels of CO_2 on the growth of *L. monocytogenes* by Morris (1995) and Lyver (1997). *L. monocytogenes* counts in raw wontons stored at 4°C are not shown due to lack of growth of these pathogens. This was attributed to high initial microbial load in raw wontons that outgrew *L. monocytogenes*, which is not a strong competitor.

L. monocytogenes grew well in most samples at moderate abuse temperature (12°C) regardless of packaging conditions (Figures 4.4-4.7). Elevated levels of CO_2 (80%) were not as effective at inhibiting the growth of *L. monocytogenes* compared to products stored at 4°C. *L. monocytogenes* grew more rapidly, and to higher levels, in all products stored at moderate temperature abuse conditions. Counts of *L. monocytogenes* in partially cooked burgers at 12°C increased from ~10^2 CFU/g to ~10^4 CFU/g within a week (Figure 4.4), but needed >21 days to reach these levels when products were stored at 4°C (Figure 4.1). Similar trends were observed in fully cooked burgers and fried wontons stored at 12°C (Figures 4.5 and 4.7). These results confirm the importance of strict temperature control in conjunction with MAP to inhibit the growth of this pathogen. The effectiveness of MAP decreased when storage temperature increased. No growth of *L. monocytogenes* was observed in raw wontons packaged under air and air + Ageless SS at 12°C (Figure 4.6). However, *L. monocytogenes* grew in gas packaged samples. This may be attributed to the inhibition of spoilage bacteria present in raw wontons by gas packaging, thus allowing the less competitive *L. monocytogenes* to grow.

Evaluations of APC, LAB, aerobic and anaerobic spore counts are not shown. *L. monocytogenes* would grow on both TSA and MRS media. This pathogen appeared as white colonies with tinted blue ring at the edges on TSA plates. As a result, the changes in APC would not provide meaningful results. For example, APC counts for both raw and fried wontons would be the same if similar numbers of colonies appeared on TSA plates that resembled spoilage bacteria and *L. monocytogenes* respectively. Similar reasons could be applied to MRS plates, while colonies of both LAB and *L. monocytogenes* were white and round. One way of determining the identity of colonies on MRS plates is by performing catalase test with 3% hydrogen peroxide. *L. monocytogenes* is catalase-positive but LAB are catalase-negative. Since the facultative *L. monocytogenes* grew on MRS plate under anaerobic incubation conditions, the results of change in LAB count were inconclusive. The counts of aerobic and anaerobic spores were too low to be recorded and thus results were not shown.

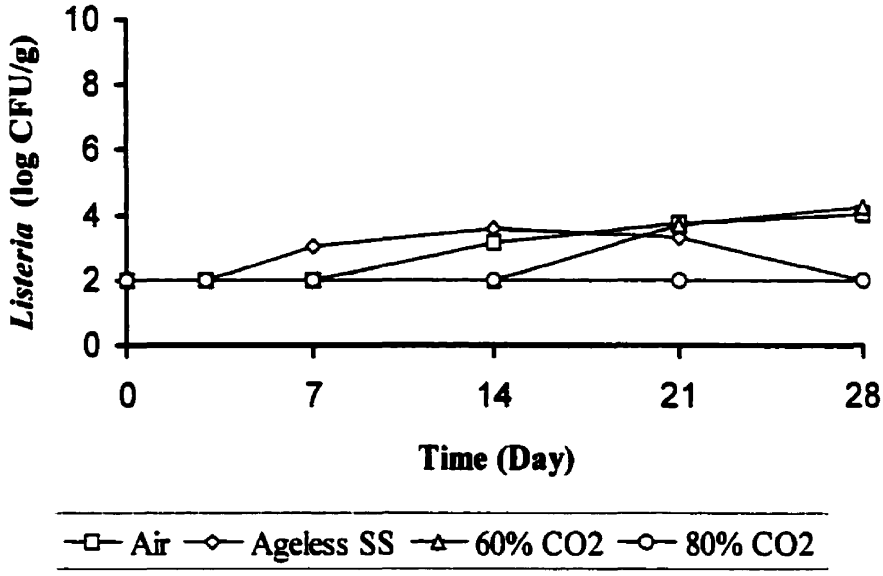

Figure 4.1: Changes in counts of *L. monocytogenes* of inoculated partially cooked burgers packaged under various gas atmospheres and stored at 4°C.

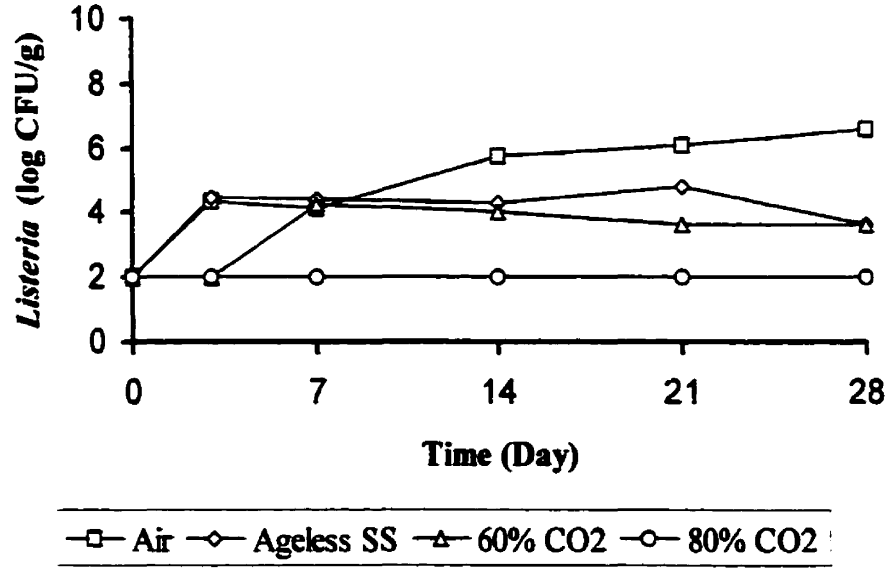

Figure 4.2: Changes in counts of *L. monocytogenes* of inoculated fully cooked burgers packaged under various gas atmospheres and stored at 4°C.

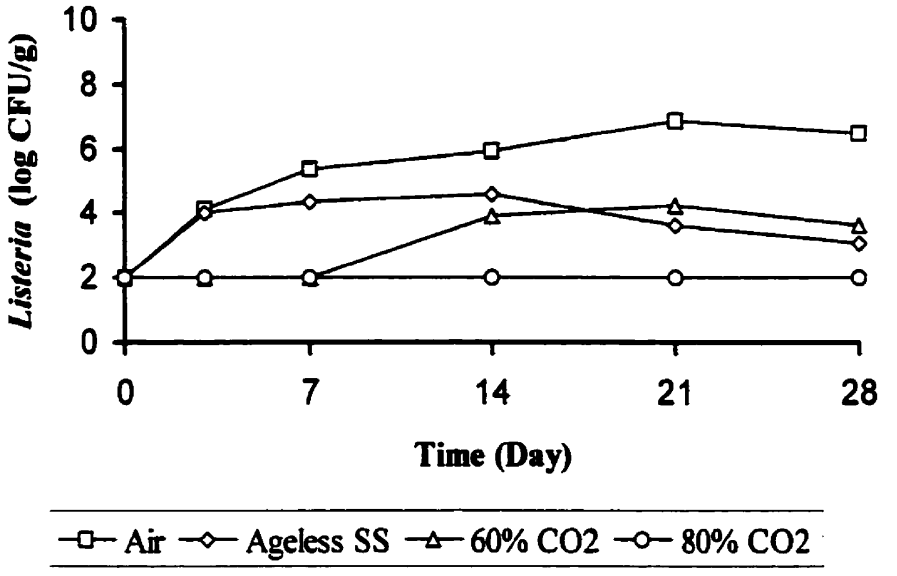

Figure 4.3: Changes in counts of *L. monocytogenes* of inoculated fried wontons packaged under various gas atmospheres and stored at 4°C.

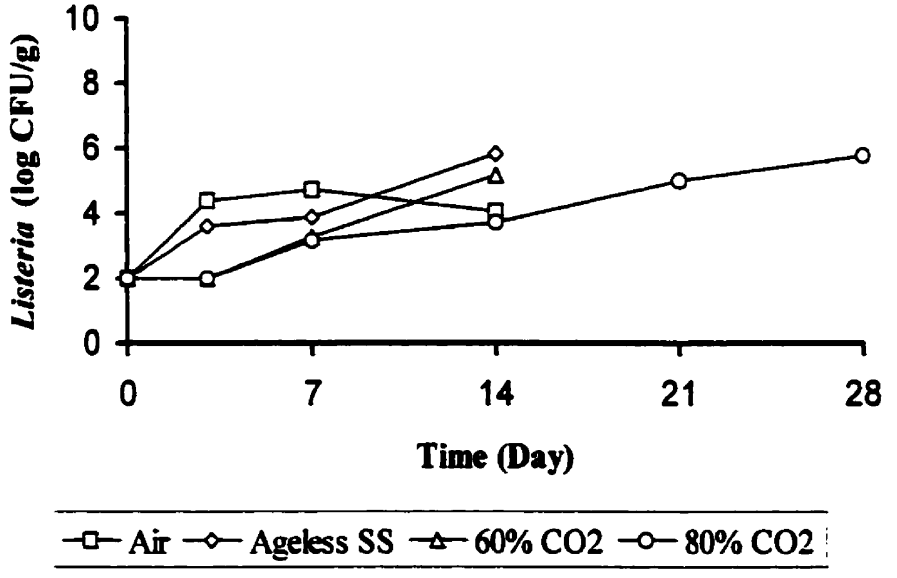

Figure 4.4: Changes in counts of *L. monocytogenes* of inoculated partially cooked burgers packaged under various gas atmospheres and stored at 12°C.

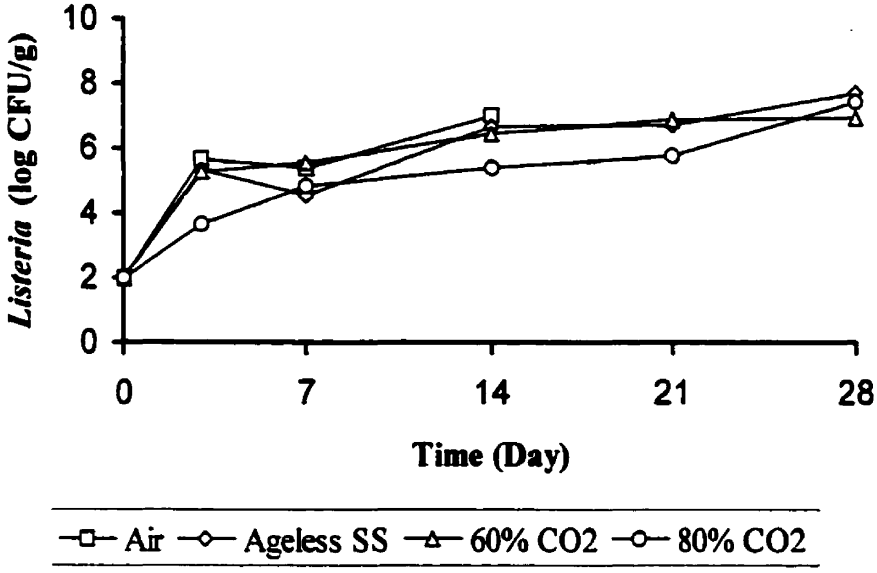

Figure 4.5: Changes in counts of *L. monocytogenes* of inoculated fully cooked burgers packaged under various gas atmospheres and stored at 12°C.

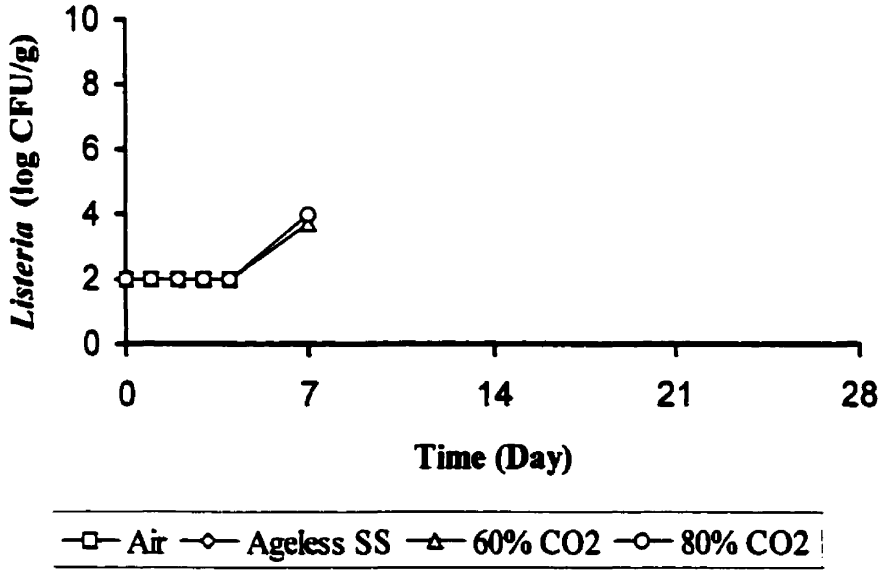

Figure 4.6: Changes in counts of *L. monocytogenes* of inoculated raw wontons packaged under various gas atmospheres and stored at 12°C.

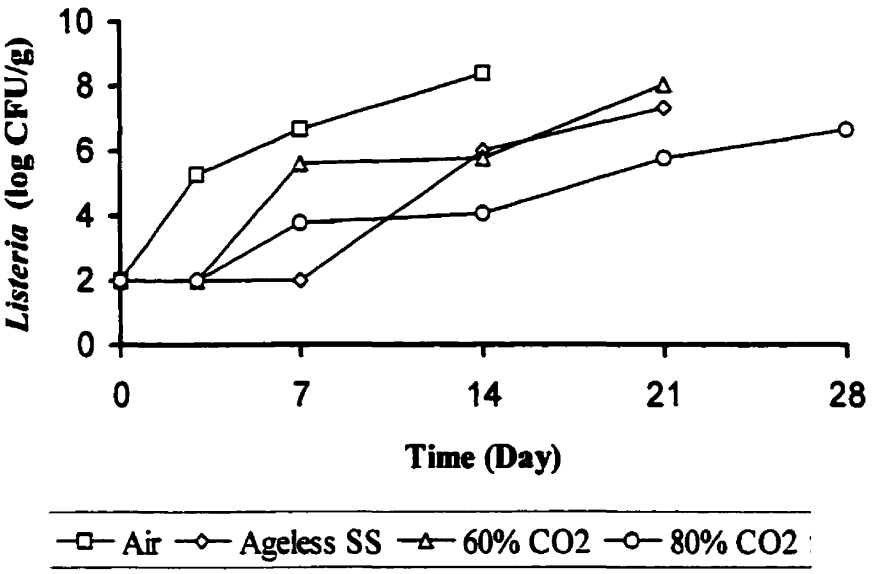

Figure 4.7: Changes in counts of *L. monocytogenes* of inoculated fried wontons packaged under various gas atmospheres and stored at 12°C.

4.3.3. Changes in pH

The changes in pH values for inoculated trout burgers and wontons are shown in Figures 4.8 to 4.15. Results of control samples (data not shown) were similar to those observed in shelf-life studies (Section 3.3.3).

The pH values for most inoculated products stored at 4°C remained fairly constant (Figures 4.8-4.11) with the exception of raw wontons. A slight increase in pH values from ~6.5 to ~7 was observed for all inoculated partially, fully cooked trout burgers and fried wontons stored at 4°C (Figure 4.8, 4.9 and 4.10). The most significant increase in pH values was observed in air packaged partially cooked burgers stored at 4°C (Figure 4.8) which increased from pH 6.6 to 7.44 after 21 days. The increase in pH may be attributed to the growth of *L. monocytogenes* in these products. Since no growth of *L. monocytogenes* was observed in inoculated raw wontons stored at 4°C, the pH values of raw wontons remained stable throughout storage.

Similar trends were observed for most products stored at 12°C, again, with the exception of raw wontons (Figure 4.14). The increases of pH values for partially, fully cooked burgers and fried wontons were not as significant (Figures 4.12, 4.13 and 4.15) compared to products stored at 4°C. A more significant decrease in pH was observed for raw wontons stored at 12°C (Figure 4.14). This observation may be attributed to higher levels of LAB found in raw wontons since these products did not undergo cooking process.

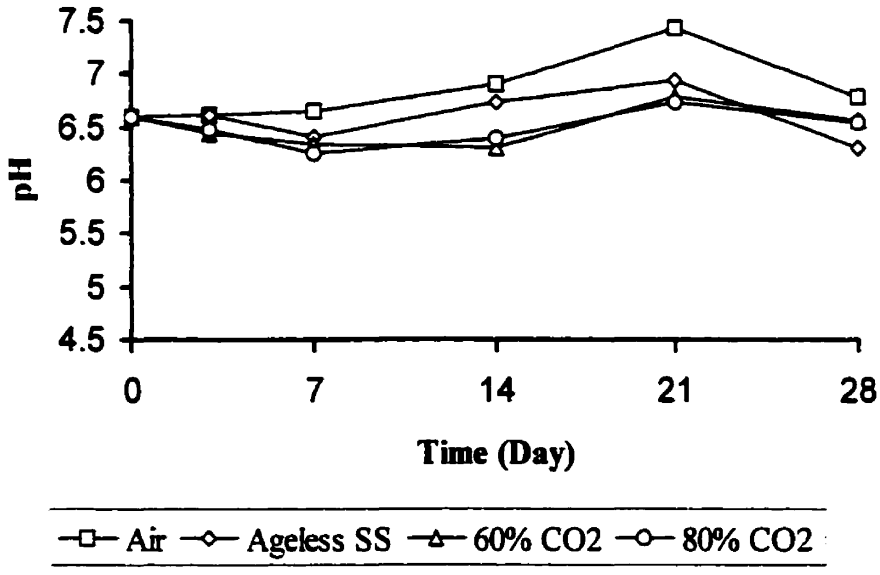

Figure 4.8: Changes in pH values of inoculated partially cooked burgers packaged under various gas atmospheres and stored at 4°C.

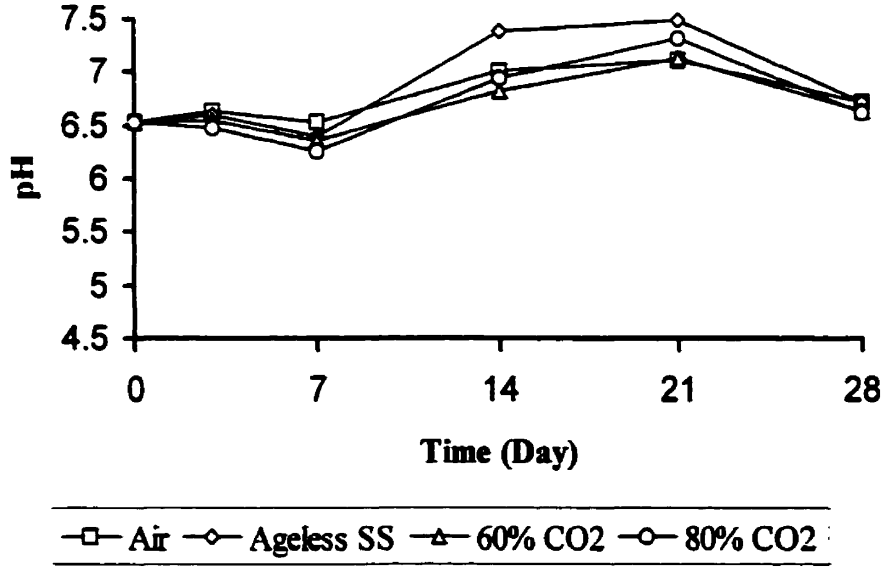

Figure 4.9: Changes in pH values of inoculated fully cooked burgers packaged under various gas atmospheres and stored at 4°C.

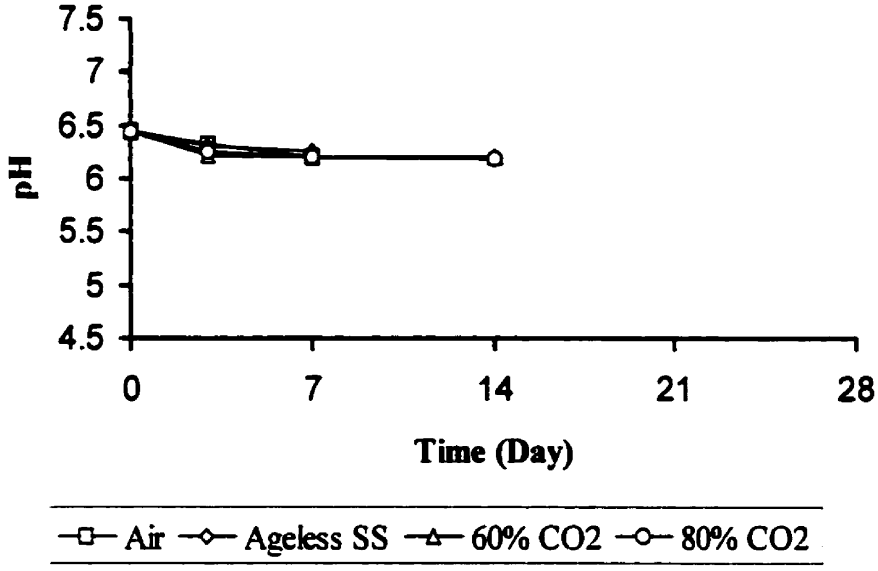

Figure 4.10: Changes in pH values of inoculated raw wontons packaged under various gas atmospheres and stored at 4°C.

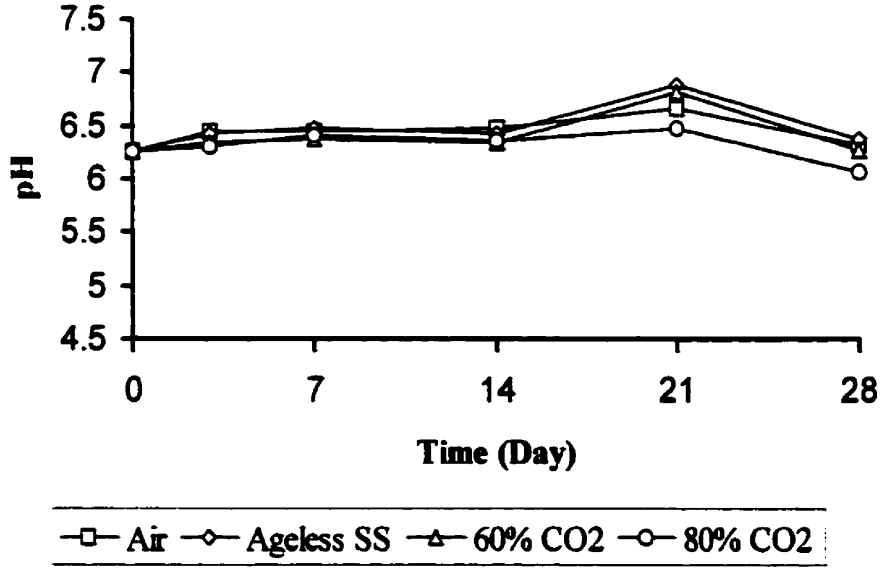

Figure 4.11: Changes in pH values of inoculated fried wontons packaged under various gas atmospheres and stored at 4°C.

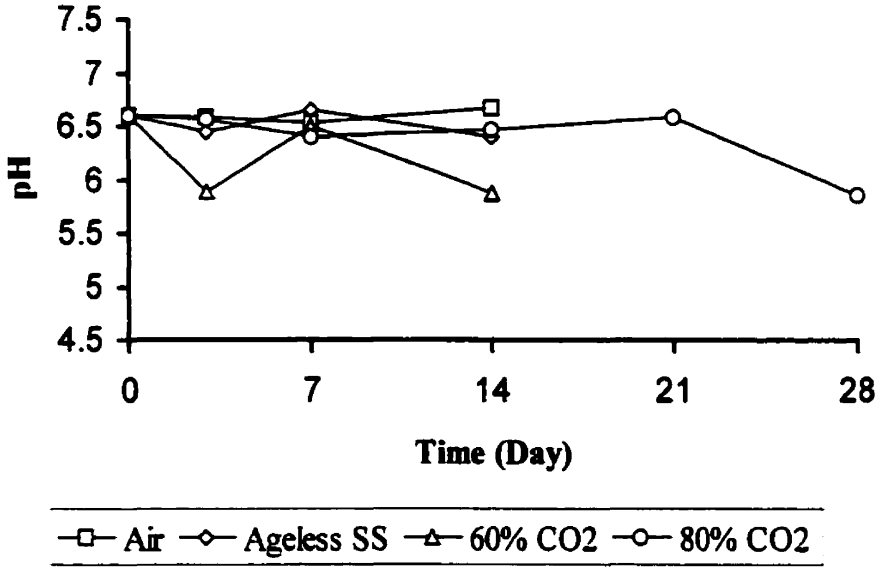

Figure 4.12: Changes in pH values of inoculated partially cooked burgers packaged under various gas atmospheres and stored at 12°C.

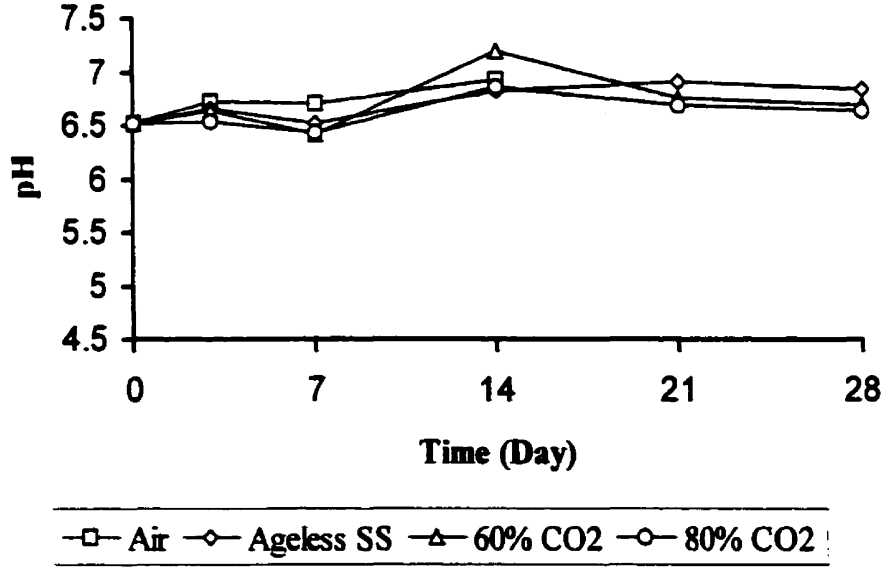

Figure 4.13: Changes in pH values of inoculated fully cooked burgers packaged under various gas atmospheres and stored at 12°C.

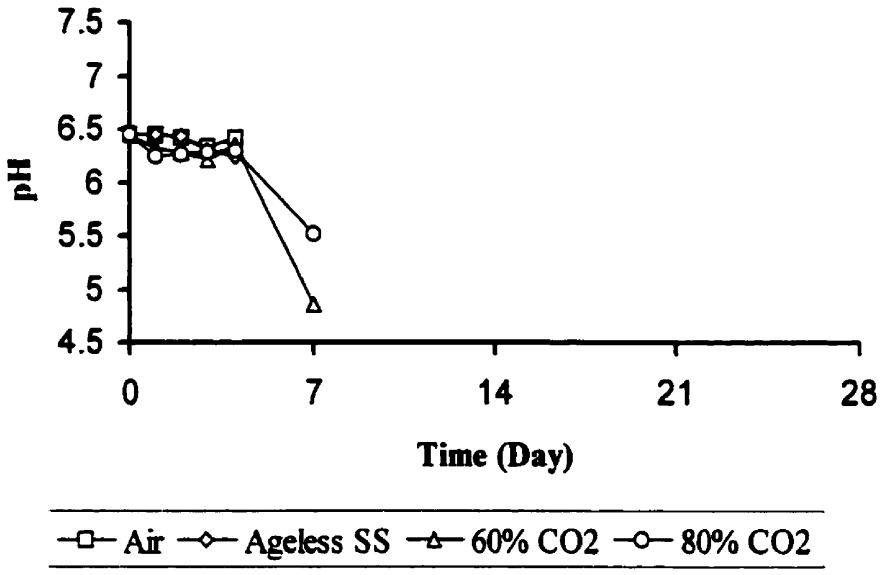

Figure 4.14: Changes in pH values of inoculated raw wontons packaged under various gas atmospheres and stored at 12°C.

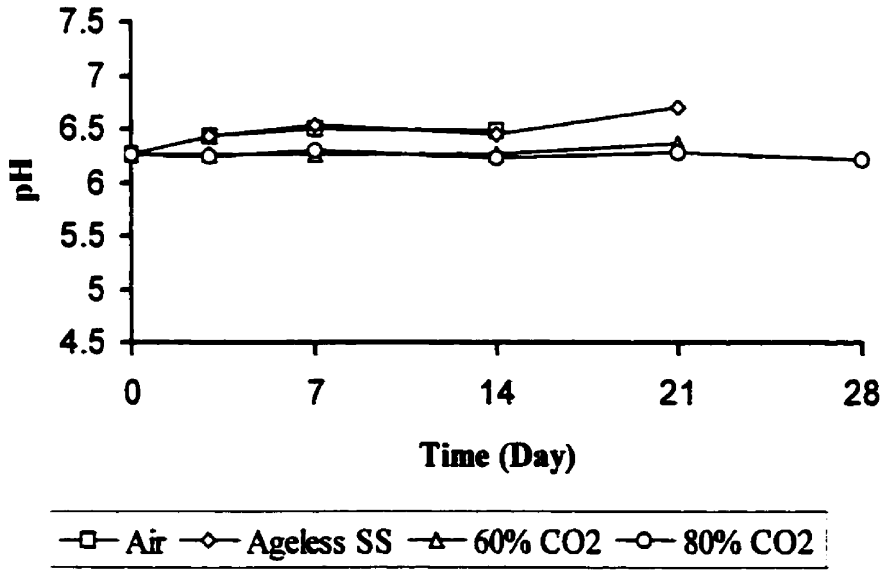

Figure 4.15: Changes in pH values of inoculated fried wontons packaged under various gas atmospheres and stored at 12°C.

4.4. Conclusions

This study has shown that there is a potential health risk associated with the formulated value-added trout products packaged MAP conditions, if contaminated with *L. monocytogenes*, since they supported the growth of this pathogen. However, the growth of *L. monocytogenes* was inhibited in 80% CO_2 packaged trout burgers and wontons stored at 4°C. These results generally agreed with those found by other authors, such as Marshall *et al.* (1991, 1992), Farber and Daley (1994), Sheridan *et al.* (1995), Morris (1995) and Lyver (1997). These studies found that an elevated CO_2 level of >70% in the package headspace could increase the lag phase, reduce the growth rate or even completely inhibit the growth of *L. monocytogenes* in various food products.

It has been shown that *L. monocytogenes* grew well in these formulated value-added trout products, even under MAP conditions. Therefore, if *L. monocytogenes* is present in the products due to inadequate cooking process or cross-contamination, the MAP value-added trout products could pose a public health risk to consumers during normal refrigerated storage. The effectiveness of MAP in inhibiting the growth of *L. monocytogenes* is further decreased when products are stored at mild temperature abuse conditions.

CHAPTER 5:
CHALLENGE STUDIES WITH *CLOSTRIDIUM BOTULINUM* TYPE E

5.1. Introduction

Clostridium botulinum type E is one of the major public health concerns with regard to safety of MAP fish and seafood products. This is due to the fact that (i) the non-proteolytic *C. botulinum* type E is widely distributed in marine and aquatic environments (Eklund *et al.*, 1984); (ii) it can grow under anaerobic conditions at refrigerated temperature (Smith and Sugiyama, 1988); (iii) the normal aerobic spoilage microorganisms are inhibited under MAP conditions (Smith, 1990b); and (iv) the growth of *C. botulinum* type E in MAP is enhanced at mild temperature abuse conditions (Garcia *et al.*, 1987).

Fresh and unprocessed seafoods usually pose few problems with regard to *C. botulinum* as spoilage by other bacteria is rapid and occurs before *C. botulinum* can grow and produce toxin. However, when seafood is processed to increase its shelf-life, such as cooking, the risk of botulism is increased. Since *C. botulinum* type E is non-proteolytic, the processed seafood products could be toxic before consumers perceived the products as spoiled when products are stored under MAP conditions. The presence of *C. botulinum* in processed seafood may due to insufficient cooking and/or cross-contamination with other food products. Recently, Lyver *et al.* (1998) reported toxin production in MAP sterile value-added shrimp nuggets by *C. botulinum* type E.

Therefore, to address concerns of the potential growth of *C. botulinum* type E in formulated value-added trout products, challenge studies were performed with this pathogen. The objectives of this study were to monitor the physical, chemical and sensorial changes in products inoculated with *C. botulinum* type E, packaged under various atmospheres and stored at refrigeration and moderate temperature abuse conditions.

5.2. Materials and Methods

5.2.1. Preparation of Samples

Value-added trout burgers and wontons were prepared as outlined in Sections 3.2.1. and 3.2.2. respectively.

5.2.2. Preparation of Bacterial Strains and Inoculation

The spore inoculum was prepared from a mixture of four *C. botulinum* type E strains (8550, Bennett, Gordon and Russ) from the culture collection of Dr. J. Austin (Bureau of Microbial Hazards, Health Protection Branch, Health Canada, Ottawa, Ontario, Canada). Cultures were grown in Trypticase Peptone Glucose Yeast Extract (TPGYE) broth at 35°C for 10 days in an atmosphere of 10% H_2, 10% CO_2 and 80% N_2 in an anaerobic chamber (Coy Laboratory Products Inc., Ann Arbor, Michigan, United States). The spores were then harvested in sterile distilled water by centrifuging at 17,500 X g for 20 min at 4°C. The spores were washed three times. The spores were then frozen at -80°C in gelatin phosphate buffer at pH 6.6 until use. Equal numbers of spores of each strain were mixed to form a single suspension of ~1 x 10^5 spores/ml. The spore mixture was heat shocked at 60°C for 20 min prior to inoculation. Each trout burger was inoculated by spraying the inoculum in three different locations (30 µl each) due to its porosity, while each trout wonton was inoculated with 15 µl of inoculum by injecting the inoculum into the fillings to give a final inoculum level of ~10^2 spore/g. Control trout products were inoculated in a similar manner using sterile gelatin phosphate buffer at pH 6.6.

5.2.3. Packaging of Samples

Partially/fully cooked trout burgers (1 per bag) and raw/fried wontons (2 per bag) were packaged in 210 mm x 210 mm high gas barrier bags (OTR: 11.6 cc/m^2/day at 24°C, 0% relative humidity (RH); Cryovac Sealed Air Corporation). Samples were packaged under air, in air with an Ageless SS-200 oxygen absorbent (Mitsubishi Gas Chemical American Inc.) and in 80% CO_2 (balance N_2). Air packaged samples were sealed using an Impulse Heat Sealer. Ageless SS-200 oxygen absorbents were inserted into the appropriate bags and sealed as described above. Gas packaging was done using a chamber type, heat seal packaging machine (Model SP-300H, Multivac Inc.). A Multivac proportional gas mixer (Model KM100-3M, Multivac Inc.) was used to give the desired proportions of CO_2 and N_2 in the package headspace. Duplicate samples of each packaging condition were stored at 4 and 12°C and were monitored for physical, chemical and microbiological changes. Partially cooked trout burgers were stored for 28 days. Raw trout wontons at 4 and 12°C were stored for 28 and 14 days respectively. Both partially cooked burgers and raw wontons were tested every 7 days. Fully cooked trout burgers and fried trout wontons were both stored for 60 days with samples being tested every 10 days.

5.2.4. Water Activity Measurement

The water activity (a_w) of samples was measured using a Decagon water activity system (Model CX-1, Decagon Devices Inc., Pullman, Washington, United States). The system was calibrated using a saturated NaCl solution (a_w 0.75 at 25°C). A_w measurements were made (in duplicate) on samples of crust, filling and mixture of crust + filling which were placed directly into plastic containers and were then inserted into the calibrated instrument.

5.2.5. Headspace Gas Analysis

Samples were analyzed for headspace gas composition using previously calibrated Mocon O_2 and CO_2 analyzers (Model HS-750 and PG-100 respectively, Mocon Ltd., Minneapolis, Minnesota, United States). Gas samples were withdrawn through a silicone septum affixed to the exterior of the bag using a 10 cc. gas-tight pressure-lock syringe (Model 790-002, Mocon Ltd.).

5.2.6. Sensory analysis

Sensory analyses were performed by 3 untrained panelists on pooled samples as described in Section 3.2.7. Only the odor of samples was evaluated since there was no noticeable change in the color and texture of products throughout storage.

5.2.7. pH measurement

The pH of samples was measured with a calibrated Fisher Acumet pH meter (Fisher Scientific). Portions of sample supernatant were transferred into clean test tubes in duplicate, and pH was measured by immersing the electrode directly into each test tube.

5.2.8. Toxin assay

All samples (~100 g/bag) were aseptically transferred into a stomacher bag and 0.1% sterile peptone water (Difco Laboratories) added to achieve a 1:3 dilution. The bag was stomached for 3 min in a Stomacher 400 LabBlender (A.J. Seward). The homogenate was then centrifuged at 11,000 rpm for 20 min at 4°C in an induction drive centrifuge (Model J2-

21M, Beckman Inc., Mississauga, Ontario, Canada). An aliquot of the sample's supernatant was extracted and filtered sterilized through a 0.45 μm Acrodisc filter (Gelman Sciences, Ann Arbor, Michigan, United States). The filtrates were trypsinized by adding 1.4% trypsin solution as 10% of total volume of extract, to activate toxin prior to mouse toxicity tests. The extracts were then incubated for 1 h at 37°C to complete the trypsinization. Duplicate white mice (20-25 g) were injected intraperitoneally with 0.5 ml of the trypsinized filtrate. Mice were observed for up to 72 hours for symptoms of botulism (pinched waist, labored breathing and/or death). Mice showing severe distress were euthanized immediately prior to actual death. Two additional mice were injected if only one mouse died. Samples were considered positive for toxin if 2/2 or ≥2/4 mice died (Hauschild *et al.*, 1975). Control samples were prepared and injected in a similar manner.

5.3. Results and Discussions

5.3.1. Changes in Water Activity

Changes in the a_w values of trout burgers and wontons are shown in Table 5.1. The a_w of most products, when freshly made and after storage, were similar with the exception of fried wontons where a_w decreased from 0.95 to 0.93 after 60 days of storage. The decrease in a_w of fried wontons was due to the migration of moisture from the fillings to the crispy, cooked and dry crust which had an initial a_w of 0.89. The low final a_w of fried wontons would likely to inhibit the growth of *C. botulinum* type E since the lowest inhibitory a_w for growth of *C. botulinum* type E is 0.95 (Baird-Parker and Freame, 1967). The a_w of all other products was conducive to the growth of *C. botulinum* type E. Despite the low a_w at the end of the storage of fried wonton. *C. botulinum* type E may have grown at the beginning of the storage and then stopped.

Table 5.1: Results for a_w analysis on various value-added trout products.

Products		A_w[1]	
		Initial	Final
Partially cooked burger[2]	Crust	0.96	0.97
	Filling	0.97	0.97
	Crust + filling	0.97	0.97
Fully cooked burger[3]	Crust	0.95	0.95
	Filling	0.95	0.95
	Crust + filling	0.95	0.96
Raw wonton[2]	Crust	0.98	0.97
	Filling	0.98	0.98
	Crust + filling	0.98	0.97
Fried wonton[3]	Crust	0.89	0.91
	Filling	0.95	0.93
	Crust + filling	0.94	0.92

[1] Results are mean values of readings from duplicate samples

[2] Final results were obtained on day 28

[3] Final results were obtained on day 60

5.3.2. Headspace Gas Analysis

Changes in headspace gas composition of control and inoculated partially and fully cooked trout burgers, and raw and fried trout wontons are shown in Table 5.2-5.5. Similar trends in headspace gas composition were observed as in shelf-life studies (Chapter 3, Section 3.3.1.). No significant difference in the gas composition was observed between control and inoculated samples. Headspace O_2 and CO_2 remained fairly constant in most samples regardless of storage temperature, with the exception of air packaged samples. Headspace O_2 of Ageless SS packaged samples decreased to <1% within three days and then increased. An increase in headspace O_2 was also observed for gas packaged samples. The increase in all of the packages may be attributed to leakage.

Changes in headspace O_2 and CO_2 of partially and fully cooked burgers showed similar trends (Table 5.2 and 5.3). No significant changes were observed in headspace gases in both products, with the exception of air packaged samples. A decrease in headspace O_2 from 21% to 6.3 % and an increase of ~42% in headspace CO_2 was observed for inoculated, partially cooked burgers stored at 12°C (Table 5.2). Similarly, headspace O_2 and headspace CO_2 of inoculated, fully cooked burgers stored at similar temperature decreased to ~0% and increased to ~39% respectively (Table 5.3). The decrease in headspace CO_2 in gas packaged samples with time can be attributed to its dissolution in the product. These results were consistent with those observed in MAP food and can be attributed to the growth and metabolism of spoilage bacteria in the products (Lambert *et al.*, 1991a,b; Lyver, *et al.*, 1998; Dufresne, 1999).

Changes in gas compositions of raw wontons and fried wontons remained stable throughout the storage (Table 5.4 and 5.5). The changes of headspace gases in both control and inoculated raw wontons were not as significant as other products due to their shorter storage period and shorter shelf-life compared to other products (Table 5.4). However, headspace CO_2 of air packaged raw wontons stored at 12°C increased ~24% in only 14 days.

No significant changes in headspace gases were observed for air packaged fried wontons stored at 12°C (Table 5.5). These results are due to the lack of microbial activity in the fully cooked products.

Table 5.2: Changes in headspace O_2 and CO_2 of control and inoculated partially cooked trout burgers packaged under various gas atmospheres and stored at 4 and 12°C for 28 days.

Temperature (°C)	Packaging condition	Headspace gas level (%v/v)			
		O_2		CO_2	
		Initial	Final	Initial	Final
		Control			
	Air	21.0	19.1	0	2.8
4	Air + Ageless SS	21.0	3.2	0	0.5
	80% CO_2	0.5	0.8	77.2	70.1
	Air	21.0	3.3	0	38.0
12	Air + Ageless SS	21.0	2.3	0	1.2
	80% CO_2	0.5	1.6	77.2	71.8
		Inoculated			
	Air	21.0	17.6	0	5.9
4	Air + Ageless SS	21.0	4.1	0	0.6
	80% CO_2	0.5	1.3	77.2	69.9
	Air	21.0	6.3	0	41.8
12	Air + Ageless SS	21.0	3.2	0	1.2
	80% CO_2	0.5	1.0	77.2	74.0

Table 5.3: Changes in headspace O_2 and CO_2 of control and inoculated fully cooked trout burgers packaged under various gas atmospheres and stored at 4 and 12°C for 60 days.

Temperature (°C)	Packaging condition	Headspace gas level (%v/v)			
		O_2		CO_2	
		Initial	Final	Initial	Final
		Control			
	Air	21.0	20.7	0	2.1
4	Air + Ageless SS	21.0	0.8	0	0.3
	80% CO_2	0.3	6.1	79.1	68.1
	Air	21.0	0.3	0	30.9
12	Air + Ageless SS	21.0	0	0	1.2
	80% CO_2	0.3	0.2	79.1	66.5
		Inoculated			
	Air	21.0	20.4	0	0.1
4	Air + Ageless SS	21.0	0.9	0	0
	80% CO_2	0.3	0.4	79.1	67.5
	Air	21.0	0.7	0	38.8
12	Air + Ageless SS	21.0	1.4	0	0.1
	80% CO_2	0.3	1.6	79.1	56.3

Table 5.4: Changes in headspace O_2 and CO_2 of control and inoculated raw trout wontons packaged under various gas atmospheres and stored at 4 and 12°C.

Temperature (°C)	Packaging condition	Headspace gas level (%v/v)			
		O_2		CO_2	
		Initial	Final	Initial	Final
		Control			
	Air	21.0	15.8	0	21.3
4[1]	Air + Ageless SS	21.0	6.8	0	0.9
	80% CO_2	0.5	0.1	77.2	79.3
	Air	21.0	16.5	0	23.4
12[2]	Air + Ageless SS	21.0	4.2	0	0.7
	80% CO_2	0.5	0	77.2	77.7
		Inoculated			
	Air	21.0	16.5	0	18.2
4[1]	Air + Ageless SS	21.0	1.3	0	0.8
	80% CO_2	0.5	0.7	77.2	76.6
	Air	21.0	14.6	0	23.8
12[2]	Air + Ageless SS	21.0	3.3	0	0.4
	80% CO_2	0.5	0.3	77.2	78.1

[1] Final results were obtained on day 28.

[2] Final results were obtained on day 14.

Table 5.5: Changes in headspace O_2 and CO_2 of control and inoculated fried trout wontons packaged under various gas atmospheres and stored at 4 and 12°C for 60 days.

Temperature (°C)	Packaging condition	Headspace gas level (%v/v)			
		O_2		CO_2	
		Initial	Final	Initial	Final
		Control			
4	Air	21.0	20.3	0	2.3
	Air + Ageless SS	21.0	0.9	0	0.3
	80% CO_2	0.3	0.4	79.1	69.6
12	Air	21.0	19.6	0	2.2
	Air + Ageless SS	21.0	0.6	0	0.2
	80% CO_2	0.3	2.3	79.1	69.7
		Inoculated			
4	Air	21.0	15.1	0	1.0
	Air + Ageless SS	21.0	0.1	0	0.2
	80% CO_2	0.3	0.5	79.1	71.4
12	Air	21.0	20.5	0	1.0
	Air + Ageless SS	21.0	0.2	0	0.1
	80% CO_2	0.3	1.2	79.1	72.5

5.3.3. Sensory Evaluation

Only odor was evaluated since other sensory attributes (color, texture and overall acceptability) showed very little change in the previous shelf-life studies (Section 3.3.4.).

Similar trends in odor scores of both control and inoculated samples were observed compared to shelf-life studies (Section 3.3.4.). No significant change in odor scores was observed between control and inoculated MAP samples. The odor scores remained acceptable (>5) for most products throughout the study (results not shown) with the exception of air packaged samples stored at 12°C. All MAP partially and fully cooked burgers had a sensory shelf-life of >28 and >60 days respectively (results not shown). All MAP raw wontons stored at 4°C had a shelf-life of >28 days, and >14 days when stored at 12°C (results not shown). MAP fried wontons had a sensory shelf-life of >60 days, regardless of storage temperature (results not shown).

The results of air packaged samples are summarized in Table 5.6. For products stored at 4°C, raw wontons appeared to have lower scores compared to other products. Odor scores of these samples decreased from 6.0 to 3.0 after 28 days. Other products stored at 4°C (partially and fully cooked burgers, and fried wontons) remained sensorially acceptable throughout the study. Odor scores of air packaged products stored at 12°C were considerably lower than those at 4°C, with the exception of fried wontons which had similar scores at both storage temperatures. The sensory shelf-life of products was significantly decreased when samples were stored at moderate temperature abuse conditions. Most air packaged products stored at this temperature (12°C) developed a sharp, musty, acidic odors when scored <3.0 on the sensory chart. This acidic odor can be attributed to the growth of LAB.

Table 5.6: Summary of odor sensory shelf-life for various air packaged inoculated value-added trout products stored at 4 and 12°C.

Temperature (°C)	Product	Odor score		Sensory shelf-life[1] (day)
		Initial	Final	
4	Partially cooked burger	7.0	4.5	28 +
	Fully cooked burger	7.0	6.0	60 +
	Raw wonton	6.0	3.0	28
	Fried wonton	7.0	6.0	60 +
12	Partially cooked burger	7.0	2.0	14
	Fully cooked burger	7.0	2.0	40
	Raw wonton	6.0	2.5	7
	Fried wonton	7.0	6.0	60 +

[1] Time (day) to reached a score of ≤3.5 based on a 7-point hedonic scale (7 = Extremely desirable, 1 = Extremely undesirable)

5.3.4. Changes in pH

Changes in pH values for inoculated partially/fully cooked trout burgers and raw/fried trout wontons packaged under various gas atmospheres and stored at 4 and 12°C are shown in Figures 5.7-5.10. It is evident that pH changed very little in partially and fully cooked burgers, and fried wontons throughout storage (Table 5.7, 5.8 and 5.10). The changes in pH values were <0.5 pH unit in these products. In fact, no significant difference in pH values was observed between products stored at 4 and 12°C. This may be attributed to the inhibition of LAB in the cooked products. The pH values of these products remained ~6.0, which is of significance since this pH was conducive to the growth of *C. botulinum* throughout storage.

Changes in pH values for inoculated raw wontons were more significant compared to other products (Table 5.9). The pH of raw wontons decreased from 6.53 to ~4.5 in both air and air + Ageless SS packaged samples. The final pH values observed for gas packaged samples were slightly >5.0 at both 4 and 12°C. Since the minimum pH for *C. botulinum* type E to grow is ~5.0, (Hauschild, 1989), the decrease of pH in raw wontons may have created unfavorable conditions for the growth of this pathogen.

The observations in this study were consistent with those reported in the challenge study of *C. botulinum* type E inoculated value-added raw and cooked shrimp nuggets by Lyver *et al.* (1998). The pH of the raw value-added seafood product decreased to a level unfavorable to the growth of *C. botulinum* type E, while the pH of cooked products remained fairly constant at 6.0, providing optimal conditions for the growth of this pathogen.

Table 5.7: Changes in pH values in inoculated partially cooked burgers packaged under various gas atmospheres and stored at 4 and 12°C for 28 days.

Temperature (°C)	Packaging condition	pH	
		Initial	Final
4	Air	6.33	6.16
	Air + Ageless SS		6.26
	80% CO_2		6.11
12	Air	6.33	5.92
	Air + Ageless SS		5.89
	80% CO_2		6.11

Table 5.8: Changes in pH values in inoculated fully cooked burgers packaged under various gas atmospheres and stored at 4 and 12°C for 60 days.

Temperature (°C)	Packaging condition	pH	
		Initial	Final
4	Air	6.43	6.32
	Air + Ageless SS		6.34
	80% CO_2		6.27
12	Air	6.43	6.51
	Air + Ageless SS		6.40
	80% CO_2		6.31

Table 5.9: Changes in pH values in inoculated raw wontons packaged under various gas atmospheres and stored at 4 and 12°C.

Temperature (°C)	Packaging condition	pH	
		Initial	Final
4[1]	Air	6.53	4.50
	Air + Ageless SS		4.52
	80% CO_2		5.25
12[2]	Air	6.53	4.41
	Air + Ageless SS		4.37
	80% CO_2		5.13

[1] Final results were obtained on day 28.

[2] Final results were obtained on day 14.

Table 5.10: Changes in pH values in inoculated fried wontons packaged under various gas atmospheres and stored at 4 and 12°C for 60 days.

Temperature (°C)	Packaging condition	pH	
		Initial	Final
4	Air	6.52	6.30
	Air + Ageless SS		6.32
	80% CO_2		6.15
12	Air	6.52	6.22
	Air + Ageless SS		6.25
	80% CO_2		6.13

5.3.5. Toxin assay

The results of the toxin assay for trout burgers and wontons packaged under different gas atmospheres and stored at 4 and 12°C are summarized in Tables 5.11-5.12. Toxin was not detected in any control samples (data not shown).

Toxin was not detected in any of the inoculated MAP partially and fully cooked burgers throughout storage, even under elevated CO_2 storage and moderate temperature abuse conditions (Table 5.11). It has been reported that toxin production was observed in fresh trout fillets stored at 12°C by *C. botulinum* type E within 5 days but not in fillets stored at 4°C (Dufresne, 1999). However, no toxin production was observed for trout burgers stored at both 4 and 12°C. The results observed in the present study agreed with those reported by Lyver (1997) where no botulinal toxin was detected in inoculated cooked shrimp nuggets. A possible explanation for the inhibition of *C. botulinum* type E in value-added cooked shrimp nuggets was the production of antimicrobial substances by substantial *Bacillus* species present in the shrimp products (Lyver, 1997). However, unlike the results observed by Lyver, *Bacillus* spp. were not detected in trout burgers throughout the studies (Sections 3.3.2.3. and 4.3.2.). Thus, the lack of growth of *C. botulinum* may be due to other intrinsic factors, such as the antimicrobial effect of spices used in trout burger formulations. bacteriocins produced by lactic acid bacteria such as *Lactococcus lactis*, *Lactobacillus plantarum* and *Pediococus pentosaceus* which have been reported to have inhibitory effect on *C. botulinum* (Crandall and Montvill, 1993). However, since LAB could be destroyed in cooked products, the latter reason is highly unlikely.

No toxin production was detected in either raw or fried wontons stored under various gas atmospheres and stored at 4 and 12°C (Table 5.12). For raw wontons, the absence of growth of, and toxin production by, *C. botulinum* type E was probably due to the production of acid by LAB and a decrease in pH to <5.0, resulting in unfavorable growth conditions. Lyver *et al.* (1998) observed similar inhibitory effects on *C. botulinum* type E in challenge

studies with raw shrimp nuggets due to the growth of LAB and reduction of pH to 4.0-4.9. The lack of toxin production by *C. botulinum* type E in fried wontons was probably due to their low a_w of <0.95 (limiting a_w for growth of *C. botulinum* type E when a_w is reduced by glycerol, Baird-Parker and Freame, 1967) after storage, thus limiting the growth of this pathogen. The low final a_w of fried wontons (~0.92) was due to the migration of moisture from the fillings to the crust of the products (Table 5.1).

Table 5.11: Results of challenge studies with value-added trout burgers packaged under various gas atmospheres and stored at 4 and 12°C.

Packaging condition	Temperature (°C)	Toxin Detection (+/-)
Partially cooked burgers[1]		
Air		-
Air + Ageless SS	4	-
80% CO_2		-
Air		-
Air + Ageless SS	12	-
80% CO_2		-
Fully cooked burgers[2]		
Air		-
Air + Ageless SS	4	-
80% CO_2		-
Air		-
Air + Ageless SS	12	-
80% CO_2		-

[1] Final results were obtained on day 28

[2] Final results were obtained on day 60

Table 5.12: Results of challenge studies with value-added trout wontons packaged under various gas atmospheres and stored at 4 and 12°C.

Packaging condition	Temperature (°C)	Toxin Detection (+/-)
	Raw wontons	
Air		-
Air + Ageless SS	4[1]	-
80% CO_2		-
Air		-
Air + Ageless SS	12[2]	-
80% CO_2		-
	Fried wontons[3]	
Air		-
Air + Ageless SS	4	-
80% CO_2		-
Air		-
Air + Ageless SS	12	-
80% CO_2		-

[1] Final results were obtained on day 28

[2] Final results were obtained on day 14

[3] Final results were obtained on day 60

5.4. Antibotulinal Roles of Background Microflora and Ingredients

5.4.1. Introduction

The inhibition of toxin production by *C. botulinum* type E in both partially and fully cooked burgers could not be explained on the basis of the a_w and pH of these products, which was conducive to the growth of this pathogen. The lack of toxin production in trout burgers may due to the antimicrobial substances produced by heat resistant background microflora in these products, and/or due to the antimicrobial activity of the ingredients.

Lyver (1997) found that *Bacillus* species survived the cooking process of value-added shrimp nuggets and inhibited the growth of *C. botulinum* type E in inoculated samples. However, toxin was produced in 14 to 28 days due to inhibition of *Bacillus* species in the sterile nuggets. Since the level of *Bacillus* species found in trout burgers was insignificant compared to Lyver's (1997) studies, it was suspected that the spices used in the formulation may have played an antimicrobial role. Inhibitory action of spices and their extracts on different microoragnisms has been reported (Ankri and Mirelman, 1999; Arora and Kaur, 1999; Fan and Chen, 1998; Naganawa *et al.*, 1996; Farag *et al.*, 1989). Ismaiel and Pierson (1990) showed that the essential oil of spices also had an inhibitory effect on the growth and germination of *C. botulinum*. Thus, spices used in the formulation of trout burgers may have contributed to the inhibition of the growth of *C. botulinum* type E.

Therefore, the objectives of this study were to determine the antibotulinal effect of ingredients used in the product formulation by conducting studies on "sterile" trout burgers, with and without spices. The possible inhibitory effect of antimicrobial substances produced by any background microflora on *C. botulinum* is eliminated using "sterile" trout burgers.

5.4.2. Materials and Methods

5.4.2.1. Preparation and packaging of samples

Two types of sterile value-added trout burgers were prepared: with and without spices, and with and without coating. Value-added trout burgers, with or without spices, were prepared as outlined in Section 3.2.1. For burgers without spices, only trout mince, oats and eggs were used to make the patty mixture. Uncoated burgers were made by scarping off the crushed cornflakes coating of trout burger using a small knife after cooking. All burgers, coated/uncoated and with/without spices, were autoclaved twice at 121°C under moist heat for 20 min to ensure proper sterilization. Samples were checked for sterility using TSA (Difco Laboratories) plates on day 0. Duplicate samples were then packaged as described in Section 5.2.3. and stored only at 12°C. All samples were tested on day 28.

5.4.2.2. Preparation of bacterial strains and inoculation

Sterile trout burgers were inoculated with 10^2 spores/g of *C. botulinum* type E as described in Section 5.2.2.

5.4.2.3. Analyses

A_w measurement, headspace gas analysis, pH measurement and toxin assay were performed as described in Sections 5.2.4., 5.2.5., 5.2.7. and 5.2.8. respectively.

5.4.2.4. Ingredient agar plate studies

To determine the antibotulinal role of ingredients in this study, each individual ground spices (cayenne pepper, garlic powder, onion flakes, spice mix and white pepper) and soy sauce, and a combination of all the spices and soy sauce were added to McClung Toabe agar supplemented with 0.5% yeast extract (Difco Laboratories) at similar levels used in the product formulation (Table 5.13). Preparation of bacterial strains was as described in Section 5.2.2. One hundred µl of 10^3 spores/ml of *C. botulinum* type E were placed on ingredient-added and control McClung Toabe agar plate in duplicate using a spread plate technique. Plates were then incubated anaerobically in an anaerobic chamber (Coy Laboratory Products Inc.) at room temperature for 7 days and then observed for growth.

Table 5.13: Levels of ingredients used in making McClung Toabe plate.

Ingredients	Level (% w/w)
Cayenne pepper	0.05
Garlic powder	2.00
Onion flakes	6.25
Soy sauce	3.50
Spice mix	6.25
White pepper	0.38

5.4.3. Results and Discussions

Sterility checks showed that no growth was observed on any TSA plates, indicating that the burgers were sterile. Results observed for control and inoculated sterile coated/uncoated value-added trout burgers, with/without spices, are shown in Tables 5.14-5.15.

Trout burgers were made with or without spices. In burgers without spices, dried onion flakes, garlic powder, white pepper, cayenne pepper, soy sauce and commercial spice mix were omitted from the formulation. However, similar cooking procedures were used to make these products. The removal of spices from the autoclaved burgers increased product a_w from 0.96 to 0.99, thus providing a more favorable environment for the growth of *C. botulinum* type E.

Trout burgers were also made coated (regular) and uncoated. The purpose of this study was to determine if the inoculation of *C. botulinum* type E spores directly into the fillings instead of spraying on top of the coating influenced toxin production. It was suspected that using the spray technique, inoculum may have remained on top of the coating without it soaking into the fillings, thus producing false negative results. However, there was no significant difference in the results between coated and uncoated samples with/without spices. This confirmed that the lack of toxin production by *C. botulinum* in trout burgers was not influenced by the method of inoculation.

The results of sterile burgers formulated with spices (regular burgers) are shown in Table 5.14. Headspace gas compositions remained constant in control samples. A decrease in headspace O_2 and an increase in headspace CO_2 in air packaged, coated and uncoated products, were observed after 28 days. However, the changes in headspace gas composition of products with spices were not as significant as those made without spices. In products without spices, headspace CO_2 increased to 17.4% while headspace O_2 decreased to zero in

air packaged burgers, regardless of their coating (Table 5.15). Since all the burgers were sterilized prior to inoculation and packaging, the CO_2 production in the inoculated samples can only be attributed to the growth and metabolism of *C. botulinum*.

The observed changes in pH between coated and uncoated products were also not significant. The pH values of inoculated burgers with spices remained fairly constant, regardless of packaging conditions (Table 5.14). However, a decrease in pH values was observed in all inoculated burgers without spices (Table 5.15). The decrease in pH can be attributed to CO_2 production by *C. botulinum* and dissolution of this CO_2 into the aqueous phase of the product. However, the final pH values observed were still >5 which is favorable to the growth of, and toxin production by, *C. botulinum* in products.

No toxin production was observed in any inoculated coated/uncoated burgers made with spices (Table 5.14), confirming previous results and eliminating the inhibitory role of any background microflora in the burgers. However, toxin was detected in all inoculated coated/uncoated trout burgers cooked without spices (Table 5.15). Samples stored under air + Ageless SS and 80% CO_2 was highly toxic, causing severe symptoms in injected mice after only 2 hours of injection. For air packaged samples, mice developed severe symptoms within 24 hours. All mice showed severe symptoms were euthanized prior to actual death.

These results indicated that the addition of spices to the product formulation influenced toxin production by *C. botulinum* type E in the trout burgers. To confirm this observation, ingredient agar plate studies were done to determine the individual and/or combined effect of spices in the formulation on the growth of *C. botulinum* type E. The results of this study are shown in Table 5.16. The ingredient agar plates had a a_w slightly higher than trout burgers (0.96-0.97), i.e. 0.98 (data not shown), which provided favorable a_w for the growth of *C. botulinum* type E. While *C. botulinum* type E grew in plates containing each individual spice and soy sauce, no growth was observed in plates containing all the spices and soy sauce. Thus, a synergistic effect of all the spices and soy sauce was

observed in this study. This synergism may be due to an increase in antimicrobial activity whenever the spices are used together. Thus, this study clearly demonstrated the powerful antibotulinal role of the spices used in the formulation of these value-added products, thereby ensuring complete inhibition of *C. botulinum* type E, even at moderate abuse storage temperature (12°C).

5.4.4. Conclusions

Toxin was detected in all sterile value-added, coated/uncoated trout burgers without spices, regardless of packaging conditions. However, no toxin was detected in any sterile burgers with spices.

Ingredient agar plate studies confirmed the effect of spices which may inhibit the growth and toxin production of *C. botulinum* type E in the MAP value-added trout burgers, thus preventing toxin production in products stored for 28-60 days, even at mild temperature abuse conditions.

Table 5.14: Summary of results on control and inoculated sterile trout burgers with spices packaged under various gas atmospheres and stored at 12°C for 28 days.

Packaging condition	A_w	Headspace gas level (%v/v)				pH		Toxin detection
		O_2		CO_2				
		Initial	Final	Initial	Final	Initial	Final	(+/-)
				Control[1]				
Air		21.0	20.5	0	0.2		6.82	-
Air + Ageless SS	0.96	21.0	0	0	0	6.29	6.60	-
80% CO_2		0	0	80.2	75.4		6.62	-
				Inoculated, coated				
Air		21.0	12.5	0	6.5		6.16	-
Air + Ageless SS	0.96	21.0	0.1	0	0	6.29	6.1	-
80% CO_2		0	0.1	80.2	71.6		5.99	-
				Inoculated, uncoated				
Air		21.0	15.9	0	3.8		6.17	-
Air + Ageless SS	0.96	21.0	0.7	0	0	6.29	6.15	-
80% CO_2		0	0.3	80.2	74.1		6.12	-

[1] Data were mean values of results obtained from coated and uncoated control burgers

Table 5.15: Summary of results on control and inoculated sterile trout burgers without spices packaged under various gas atmospheres and stored at 12°C for 28 days.

Packaging condition	A_w	Headspace gas level (%v/v)				pH		Toxin detection
		O_2		CO_2				
		Initial	Final	Initial	Final	Initial	Final	(+/-)
				Control[1]				
Air		21.0	19.3	0	0		6.40	-
Air + Ageless SS	0.99	21.0	0	0	0	6.68	6.79	-
80% CO_2		0	0	80.2	74.6		6.63	-
				Inoculated, coated				
Air		21.0	0	0	17.4		6.47	+
Air + Ageless SS	0.99	21.0	0	0	0	6.68	6.27	+
80% CO_2		0	0	80.2	71.1		6.06	+
				Inoculated, uncoated				
Air		21.0	1.0	0	17.4		6.47	+
Air + Ageless SS	0.99	21.0	0	0	2.9	6.68	6.15	+
80% CO_2		0	0	80.2	71.7		6.10	+

[1] Data were mean values of results obtained from coated and uncoated control burgers

Table 5.16: Effect of spices and soy sauce, alone or in combination with each other, on growth of *C. botulinum* type E on McClung Toabe agar plate stored for 7 days at room temperature.

Ingredient	Growth of *C. botulinum* type E
Cayenne pepper	+
Garlic powder	+
Onion flakes	+
Soy sauce	+
Spice mix	+
White pepper	+
All spices + soy sauce	-

5.5. Conclusions

Toxin production by *C. botulinum* type E was inhibited in partially and fully cooked trout burgers due to the antimicrobial activity of the spices used in the formulation.

Toxin production was inhibited in raw wontons due to their low final pH of <5 (minimum pH for the growth of *C. botulinum* type E) caused by the growth of LAB, the predominant spoilage bacteria found in the product, and/or the antimicrobial substances in the ingredients.

Toxin production was inhibited in fried wontons probably due to moisture migration from the fillings to the crust resulting in a final a_w of <0.95 (limiting a_w for *C. botulinum* to grow) in the fillings, and/or the antimicrobial activity of the spices.

GENERAL CONCLUSIONS

The seafood processing industry is an important component of the Canadian food industry, generating employment and billions of dollars in revenue. However, with increasing environmental concerns about dumping of fish wastes and dwindling of fish stocks, it is critical to examine more profitable methods of utilizing seafood processing waste as well as under-utilized fish species. One method is to produce value-added convenience seafood products.

This study had shown that good quality, microbiological and consumer acceptable value-added trout products can be made using trout trimmings waste. Two new products were created: value-added trout burgers and wontons. Sensory evaluation of these products indicated that these formulated value-added products from trout trimmings were highly acceptable and had a tremendous market potential. Thus, this study has shown that, with some modification, an otherwise discarded material could be transformed into a more profitable product. The new product created would not only address environmental concerns of dumping of seafood processing wastes, but also lower the cost of disposal and turn a waste material into profit.

Traditionally, value-added products have been marketed frozen to ensure a longer shelf-life. However, with increasing consumer demands for "fresh" products, and increasing energy costs associated with freezing and frozen storage, modified atmosphere packaging is considered one of the alternative methods in economizing energy costs while extending the shelf-life of products.

The potential of MAP (gas packaging and oxygen absorbent technology) in extending the shelf-life of the newly formulated value-added trout products has been investigated. Under strict temperature control (refrigeration temperature at 4°C), both MAP trout burgers and fried wontons had a microbiological shelf-life of > 28 days. Even raw wontons, which

had a high initial microbial load, product shelf-life could be extended for more than a week at 4°C compared to air packaged samples. However, the shelf-life of MAP raw wontons decreased when stored under moderate temperature abuse conditions (12°C). The effectiveness of MAP is not only dependant on temperatures but also the initial microbial load of products. Since fully cooked trout burgers and fried wontons had a longer cooking processes, less microorganisms could survive the cooking process, and thus cooked products, as expected, would have a longer expected shelf-life under MAP conditions. A shelf-life of > 28 days could also be achieved when these products were stored at moderate temperature abuse conditions under MAP. MAP also extended the sensorial shelf-life of products, which was usually longer than the microbiological shelf-life. Furthermore, gas packaging, with high levels of CO_2, was found to be slightly more effective than oxygen absorbents to extend shelf-life of the products stored under refrigeration temperature.

While MAP can be used to extend the shelf-life and keeping quality of the formulated value-added trout products, there are concerns about the microbiological safety of such products, particularly with respect to the growth of *L. monocytogenes* and *C. botulinum* type E at mild temperature abuse storage conditions. Challenge studies were performed with these two pathogens to address these safety concerns.

Challenge studies with *L. monocytogenes* showed that this pathogen could grow at both 4 and 12°C, with 12°C being a more favorable condition for growth. Only gas packaged products with 80% CO_2 (balance N_2) and stored at 4°C did not support the growth of this pathogen. When storage temperature increased, *L. monocytogenes* grew in all products, regardless of gas atmosphere. The presence of this pathogen in the MAP products would therefore pose a public health risk. However, the risk would appear minimal since *L. monocytogenes* was not detected in any of the control samples. An interesting observation was that *L. monocytogenes* did not grow well in raw wontons, probably due to microbial competition. *L. monocytogenes* only grew in gas packaged raw wontons stored at 12°C and the level was significantly lower than the growth in other products stored under similar

conditions.

No botulinal toxin was detected in any of the MAP products inoculated with *C. botulinum* type E, even at mild storage temperature (12°C). The marginal a_w of the trout burgers and/or the use of spices were suspected to inhibit the growth of *C. botulinum* type E. The low final pH (< 5) of raw wontons, due to the growth of LAB, could also have inhibited the growth of *C. botulinum* type E in this product. The low final a_w (< 0.95) of fried wonton after storage could have contributed to the inhibition of the growth of the pathogen. It would appear that the growth of *C. botulinum* type E, and thus the production of toxin, in the formulated value-added products was highly unlikely, even at storage temperature abuse conditions.

In conclusion, the utilization of trout processing wastes and MAP offers the food industry a viable method of processing and storage of the formulated value-added trout products. Furthermore, the products would appear to be safe with respect to the growth of *C. botulinum* type E. However, the products did support the growth of *L. monocytogenes*. Thus, there could be a public health concern if these products were contaminated with *L. monocytogenes*.

REFERENCES

Abeyta, C. Jr., Kaysner, C.A., Wekell, M.M., Sullivan, J.J. and Stelma, G.N. (1986). Recovery of *Aeromonas hydrophila* from oysters implicated in an outbreak of foodborne illness. *J. Food Protect.* **49**:643-646.

Aichin, C.C. and Thomas, R.C. (1975). Micro-electrode measurement of the internal pH of crab muscle fiber. *J. Physiol.* **52**:803-807.

Ajmal, M. (1968). Growth and toxin production of *Clostridium botulinum* type E. *J. Appl. Bacteriol.* **31**:120-123.

Ankri, S. and Mirelman, D. (1999). Antimicrobial properties of allicin from garlic. *Microbes & Infection.* **1**:125-129.

Arora, D.S. and Kaur, J. (1999). Antimicrobial activity of spices. *Int. J. Antimicrobial Agents.* **12**:257-262.

Ashie, I.N.A., Smith J.P. and Simpson, B.K. (1996). Spoilage and shelf-life extension of fresh fish and shellfish. *Crit. Rev. Food Sci. & Nutr.* **36**:87-121.

Avery, J.J.W. and Lamprecht, A. (1988). The shelf-life extension of fresh hake through gamma irradiation. *Food Rev.* **15** (suppl.):28.

Avery, S.M., Hudson, J.A. and Penney, N. (1994). Inhibition of *Listeria monocytogenes* on normal ultimate pH beef (pH 5.3-5.5) at abusive storage temperatures by saturated carbon dioxide controlled atmosphere packaging. *J. Food Protect.* **57**:331-336.

Babbitt, J.K. (1986). Suitability of seafood species as raw materials. *Food Technol.* **40**(3):97-100.

Bailey, J.S., Fletcher, D.L. and Cox, N.A. (1990). *Listeria monocytogenes* colonization fo broiler chicken. *Poultry Sci.* **69**:457-461.

Baird-Parker, A.C. and Freame, B. (1967). Combined effect of water activity, pH, and temperature on the growth of *Clostridium botulinum* from spore and vegetative cell inocula. *J. Appl. Bacteriol.* **30**:420-429.

Banwart, F.J. (1989). *Basic Food Microbiology.* AVI Publishing, Connecticut, U.S.A.

Barefoot, S.F., Harmon, K.M., Grinstead, D.A. and Nettles, C.G. (1992). Bacteriocins, molecular biology. In: Lederberg, J. (ed.) *Encyclopedia of Microbiology, Vol. I.* Academic Press Inc., New York, U.S.A. pp. 191-202.

Becker, Z.E. (1933). A comparison between the action of carbonic acid and other acids upon the living cell. *Protoplasma.* **25**:161-175.

Beuchat, L.R., Berrang, M.E. and Brackett, R.E. (1990). Presence and public health implication of *Listeria monocytogenes* on vegetables. In: Miller, A.J., Smith, J.L. and Somkuti, G.A. (eds.) *Foodborne Listeriosis.* Elsevier Science Publishing Inc., New York, U.S.A. pp. 175-181.

Blewett, E. and MacDonald, J.D. (1998). Potential impacts of patenting life forms on the aquatic products sector in Canada. *Intellectual Property Policy Directorate of Industry Canada.*

Bligh, E.G. and Duclos-Rendell, R. (1986). Chemical and physical characteristics of lightly salted mined cod (*Gadus morhua*). *J. Food Sci.* **51**:76-78.

Bramstedt, F. and Auerbach, M. (1961). The spoilage of fresh-water fish. In: Borgstrom, G. (ed.) *Fish as Food.* Academic Press, New York, U.S.A. pp. 613-637.

Brinkman, R., Margaria, R. and Roughton, F.J.W. (1933). The kinetics of the carbon dioxide-carbonic acid reaction. *Proc. R. Soc. Lon.* **232**:65-73.

Brody, A.L. (1989). Modified atmosphere packaging of seafoods. *Controlled/Modified Atmosphere Packaging of Foods.* Food and Nutrition Press Inc., Connecticut, U.S.A. pp. 59-66.

Callow, E.H. (1932). Gas storage of pork and bacon: part I — preliminary experiments. *J. Soc. Chem. Indust.* **51**:116-119T.

Campden Technical Manual. (1992). *Guidelines for the Good Manufacturing and Handling of Modified Atmosphere Packed Products.* Campden Food and Drink Association, Gloucestershire, U.K.

Canadian Department of Fisheries. (1959). *Canadian Fish Cook Book: A Handbook on How to Buy, Prepare, and Serve All Kinds of Canadian Fish and Shellfish at Every Season of the Year.* Minister of Fisheries, Ontario, Canada. pp. 51,77.

Cann, D.C. (1984). Packing fish in a modified atmosphere. *Torry Advisory Note.* No. 88.

Castelli, A., Littaru, G.P. and Barbesi, G. (1969). Effect of pH and CO_2 concentration changes on lipids and fatty acids of *Saccharomyces cerevisiae. Arch. Mikrobiol.* **66**:34-39.

Christiansen, L.N. (1980). Factor influencing botulinal inhibition by nitrite. *Food Technol.* **34**:237-239.

Christiansen, L.N. and Foster, E.M. (1965). Effect of vacuum packaging on growth of *Clostridium botulinum* and *Staphylococcus aureus* in cured meats. *Appl. Microbiol.* **13**:1023-1025.

Collins-Thompson, D.L. and Wood, D.S. (1993). Control in dairy products. In: Hauschild, A.H.W. and Dodds, K.L. (eds.) *Clostridium botulinum: Ecology and Control in Foods.* Marcel Dekker Inc., New York, U.S.A. pp. 261-277.

Crandall, A.D. and Montville, T.J. (1993). Inhibition of *Clostridium botulinum* growth and toxigenesis in a model gravy system by coinoculation with bacteriocin-producing lactic acid bacteria. *J. Food Protect.* **56**:485-488.

Daeshel, M.A. (1989). Antimicrobial substances produced from lactic acid bacteria for use as food preservatives. *Food Technol.* **43**:164-167.

Delente, J., Akin, C., Krabbe, E. and Ladenburg, K. (1969). Fluid dynamics of anaerobic fermentation. *Biotechnol. & Bioeng.* **11**:631-646.

Dempster, J.F. (1985). Radiation preservation of meat and meat products – a review. *Meat Sci.* **12**:61-69.

Devlieghere, F., Lefevere, I. and Debevere, J. (1998). Growth of *Listeria monocytogenes* and *Aeromonas hydrophila* under modified atmospheres: a predictive model. Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent. University Gent: Gent, Belgium. **63**:1439-1446.

Dodds, K.L. (1994). *Clostridium botulinum.* In: Hui, Y.H., Gorham, J.R., Murrell, K.D. and Cliver, D.O. (eds.) *Foodborne Disease Handbook.* Marcel Dekker Inc., New York, U.S.A. pp. 105-117.

Doherty, A., Sheridan, J.J., Allen, P., McDowell, D.A., Blair, I.S. and Harrington, D. (1996). Survival and growth of *Aeromonas hydrophila* on modified atmosphere packaged normal and high pH lamb. *Int. J. Food Microbiol.* **28**:379-392.

Dolye, M.P., Glass, K.A., Berry, J.T., Garcia, G.A., Pollard, D.J. and Schultz, R.D. (1987). Survival of *Listeria monocytogenes* in milk during high temperature, short time pasteurization. *Appl. Env. Microbiol.* **53**:1433-1438.

Dufresne, I. (1999). *M. Sc. Thesis: Shelf-life and safety studies on rainbow trout fillets packaged under modified atmospheres.* McGill University, Quebec, Canada.

Eddy, B.P. and Ketchell, A.G. (1959). Cold-tolerant fermentative gram-negative organisms from meat and other sources. *J. Appl. Bacteriol.* **22**:57-63.

Eklund, M.W. (1982). Significance of *Clostridium botulinum* in fishery products preserved short of sterilization . *Food Technol.* **36**(12):107-112.

Eklund, M.W. (1993). Control in fishery products. In: Hauschild, A.H.W. and Dodds, K.L. (eds.) *Clostridium botulinum: Ecology and Control in Foods.* Marcel Dekker Inc. New York, U.S.A. pp. 209-232.

Eklund, M.W. and Poysky, F.T. (1965). *Clostridium botulinum* type E from marine sediments. *Science* **149**:306.

Eklund, M.W. and Poysky, F.T. (1967). Incidence of *C. botulinum* type E from the Pacific coast of the United States. In: Ingram, M. and Roberts, T.A. (eds.) *Botulinum 1966.* Chapman and Hall, London, U.K. pp. 49-55.

Eklund, M.W., Poysky, F.T., Peterson, M.E., Peck, L.W. and Brunson, W.D. (1984). Type E botulism in salmonids and conditions contributing to outbreaks. *Aquaculture.* **41**:293-309.

El-Kest, S.E., Yousef, A.E. and Marth, E.H. (1991). Fate of *Listeria monocytogenes* during freezing and frozen storage. *J. Food Sci.* **56**:1068-1071.

El-Shenawy, M.A. and Marth, E.H. (1988). Sodium benzoate inhibits growth or inactivates *Listeria monocytogenes. J. Food Protect.* **51**:525-530.

El-Zeany, B.A., Polorny, J.A. and Janicek, G. (1975). Autoxidation of fish oil fatty acid esters in mixture with protein in presence of copper and iron salts. *Inst. of Chem. Tech. Prague E.* **42**:5.

Elsden, S.R. (1938). The effect of carbon dioxide on the production of succinic acid by *Bacillus* spp. *Biochem. J.* **32**:187-193.

Enfors, S.O. and Molin, G. (1978). The influence of high concentrations of carbon dioxide on the germination of bacterial spores. *J. Appl. Bacteriol.* **4**:279-285.

Erdman, I.E., Thatcher, F.S. and MacQueen, K.F. (1961). Studies on the irradiation of microorganisms in relation to food preservation, part I – the comparative sensitivities of specific bacteria of public health significance. *Can. J. Microbiol.* **7**:199-205.

Fan, J. and Chen, J. (1998). Antimicrobial activity of welsh onion ethanol extracts. *J. Chinese Agr. Chem. Soc.* **36**:12-20.

Farag, R.S., Daw, Z.Y., Hewedi, F.M. and El-Baroty, G.S.A. (1989). Antimicrobial activity of some Egyptian spice essential oils. *J. Food Protect.* **52**:665-667.

Farber, J.M. (1991). Microbiological aspects of modified atmosphere packaging technology – a review. *J. Food Protect.* **54**:58-70.

Farber J.M. and Brown, B.E. (1990). Effect of prior heat shock on heat resistance of *Listeria monocytogenes* in meat. *Appl. Env. Microbiol.* **56**:1584-1587.

Farber, J.M. and Daley, E. (1994). Fate of *Listeria monocytogenes* on modified atmosphere packaged turkey roll slices. *J. Food Protect.* **57**:1098-1100.

Farber, J.M., Coates, F. and Daley, E. (1992). Minimal water activity requirements for the growth of *Listeria monocytogenes*. *Letters in Appl. Microbiol.* **15**:103-105.

Farber, J.M. and Peterkin, P.I. (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbiol Rev.* **55**:476-511.

Fleming, D.W., Cochi, S.L., MacDonald, K.L., Brondum, J., Hayes, P.S., Plikaytis, B.D., Holmes, M.B., Audurier, A., Broome, C.V. and Reingold, S.L. (1985). Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *N. Engl. J. Med.* **312**:404-407.

Flick, G.J. and Lovell, R.T. (1972). Postmortem biochemical changes in the muscle of gulf shrimp, *Penaeus aztecus*. *J. Food Sci.* **37**:609-611.

Fox, D.L. (1981). Presumed carbaminoprotein equilibria and free energy exchanges in reversible carbon dioxide narcosis of protoplasm. *J. Theoret. Bio.* **90**:441-443.

Fox, J.B. Jr. and Nicholas, R.A. (1974). Nitrite in meat: effect of various compounds of loss of nitrite. *J. Agric. Food Chem.* **22**:302-306.

Fung, D.Y.C. (1983). The microbiology of batter and breading. In: Suderman, D.R. and Cunningham F.E. (eds.) *Batter and Breading.* AVI Publishing, Connecticut, U.S.A. pp. 106-119.

Garcia, G.W., Genigeorgis, C. and Lindroth, S. (1987). Risk of growth and toxin production by *Clostridium botulinum* nonproteolytic types B, E, and F in salmon fillets stored under modified atmospheres at low and abused temperatures. *J. Food Protect.* **50**:330-336.

Gill, C.O. and Reichel, M.P. (1989). Growth of the cold-tolerant pathogens Yersinia enterocolitica, Aeromonas hydrophila, and *Listeria monocytogenes* on high-pH beef packaged under vacuum or carbon dioxide. *Food Microbiol.* **6**:223-230

Gill, C.O. and Tan, K.H. (1980). Effect of carbon dioxide on meat spoilage bacteria. *Appl. Env. Microbiol.* **39**:317-324.

Gillespie, N.C. and Macrae, I.C. (1975). The bacterial flora of some Queensland fish and its ability to cause spoilage. *J. Appl. Bacteriol.* **32**:91-100.

Graham, A.F., Mason, D.R., Maxwell, F.J., and Peck, M.W. (1997). Effect of pH and NaCl on growth from spores of non-proteolytic Clostridium botulinum at chill temperature. *Letters Appl. Microbiol.* **24**:95-100.

Gray, M.L. and Killinger, H.A. (1966). *Listeria monocytogenes* and *Listeria* infections. *Bacteriol. Rev.* **30**:309-382.

Grecz, N. and Arvay, L.H. (1982). Effect of temperature on spore germination and vegetative cell growth of *Clostridium botulinum*. *Appl. Env. Microbiol.* **43**:331-337.

Grecz, N., Walker, A.A., Anelis, A. and Berkowitz, D. (1971). Effect of irradiation temperature in the range -196 to 95°C on the resistance of spores of *Clostridium botulinum* 33A in cooked beef. *Can. J. Microbiol.* **17**:135-142.

Greer, G.G. (1993). Estimation of chilled meat storage life. Agriculture Canada Research Branch. Lacombe Research Station, Lacombe, Alberta, Canada.

Hangard-Vidaud, N., Nicolas, J.A., Bosgiraud, C. and Cornuejols, M.J. (1989). Recherche de *Listeria* chez les poissons d'eau douce. *Microbiol. Alim. Nutr.* 7:421-423.

Hatheway, C.L. (1993). *Clostridium botulinum* and other Clostridia that produce botulinum neurotoxin. In: Hauschild, A.H.W. and Dodds, K.L. (eds.) *Clostridium botulinum: Ecology and Control in Foods.* Marcel Dekker Inc., New York, U.S.A. pp. 3-20.

Hauschild, A.H.W. (1989). *Clostridium botulinum.* In: Doyle, M.P. (ed.) *Foodborne Bacterial Pathogens.* Marcel Dekker, Inc., New York, U.S.A. pp. 111-189.

Hauschild, A.H.W., Aris, B.J. and Hilsheimer, R. (1975). *Clostridium botulinum* in marinated products. *Can. Int. Food Sci. Technol. J.* **8**:84-87.

Heisick, J.E., Wagner, D.E., Nierman, M.I. and Peeler, J.T. (1989). *Listeria* spp. found on fresh market produce. *Appl. Env. Microbiol.* **55**:1925-1927.

Herbert, A. and Shewan, J.M. (1976). Roles played by bacteria and autolytic enzymes in the production of volatile sulfides in spoiling North Sea cod. *J. Sci. Food Agric.* **27**:89-94.

Howarth, A.J. (1983). *The Canadian Fish Cook Book.* Douglas & McIntyre, British Columbia, Canada. pp. 116,180.

Hurst, A. (1981). Nisin. *Adv. Appl. Microbiol.* **27**:85-123.

International Commission on Microbiological Specifications for Foods (ICMSF). (1996). Microbiological specifications of food pathogens. In: ICMSF (ed.) *Microorganisms in Foods*. Blackie Academic & Professional, London, U.K.

Ingham, S.C. (1991). Microbiology of mince, surimi, and value-added seafoods. In: Ward, D.R. and Hackney, C. (eds.) *Microbiology of Marine Food Products.* Van Nostrand Reinhold, New York, U.S.A. pp. 89-110.

Ingham, S.C. and Potter, N.N. (1988). Survival and growth of *Aeromonas hydrophila, Vibrio parahaemolyticus*, and *Staphylococcus aureus* on cooked mince and surimis made from Atlantic pollock. *J. Food Protect.* **51**:634-638.

Ismaiel, A. and Pierson. M.D. (1990). Inhibition of growth and germination of *C. botulinum* 33A, 40B and 1623E by essential oil of spices. *J. Food Sci.* **55**:1676-1678.

Ita, P. and Hutkins, R.W. (1991). Intracellular pH and survival of *Listeria monocytogenes* Scott A in tryptic soy broth containing acetic, lactic, citric, and hydrocholoric acids. *J. Food Protect.* **54**:15-19.

Jay, J.M. (1983). Antibiotics as food preservatives. In: Rose, A.H.. (ed.) *Food Microbiology.* Academic Press, London, U.K. p. 117.

Johnson, J.L., Doyle, M.P. and Cassens, R.G. (1990). Incidence of *Listeria* spp. in retail meat roasts. *J. Food Sci.* **55**:572-574.

Kafel, S. and Ayres, J.C. (1969). The antogonism of Enterococci on other bacteria in canned hams. *J. Appl. Bacteriol.* **32**:217-232.

Khayat, A. and Schwall, D. (1983). Lipid oxidation in seafood. *Food Technology.* **37**:130-140.

Kimura, B., Murakami, M. and Fujii, T. (1997). Growth of selected food spoilage and pathogenic bacteria under modified atmosphere. *Fisheries Sci.* **63**:1030-1034.

Kimura, B., Yoshiyama, T. and Fujii, T. (1999). Carbon dioxide inhibition of *Escherichia coli* and *Staphylococcus aureus* on a pH-adjusted surface in a model system. *J. Food Sci.* **64**:367-370.

King, A.D. and Nagel, C.W. (1967). Growth inhibition of a *Pseudomonas* by carbon dioxide. *J. Food Sci.* **32**:575-579.

King, A.D. and Nagel, C.W. (1975). Influence of carbon dioxide upon the metabolism of *Pseudomonas aereuginosa*. *J. Food Sci.* **40**:362-366.

Klaenhammer, T.R. (1988). Bacteriocins of lactic acid bacteria. *Biochemie.* **10**:337-349.

Knable, S.J., Walker, H.W., Hartman, P.A. and Mendonca, A.F. (1990). Effects of growth temperature and strictly anaerobic recovery on the survival of *Listeria monocytogenes* during pasteurization. *Appl. Env. Microbiol.* **56**:370-376.

Knoche, W. (1980). Chemical reactions of CO_2 in water. In: Bauer, C., Gros, G. and Bartels, H. (eds.) *Biophysics and Physiology of Carbon Dioxide*. Springer-Verlag, Berlin, Germany. pp. 3-11.

Krebs, H.A. and Roughton, R.J.W. (1948). Carbonic anhydrase as a tool in studying the mechanisms of reactions involving H_2CO_3, CO_2 or HCO_3. *Biochem. J.* **43**:550-555.

Krough, A. (1919). The rate of diffusion of gases through animal tissues with some remarks on the coefficient of invasion. *J. Physiol.* **52**:391.

Kvenberg, J.E. (1991). Nonindigenous bacterial pathogens. In: Ward, D.R. and Hackney, C. (eds.) *Microbiology of Marine Food Products.* Van Nostrand Reinhold, New York, U.S.A. pp. 267-284.

Lambert, A. (1991a). *Ph.D. Thesis: Effects of modified atmosphere packaging and low-dose irradiation on the shelf-life and microbiological safety of fresh pork.* McGill University, Quebec, Canada.

Lambert, A.D., Smith, J.P. and Dodds, K.L. (1991b). Combined effects of modified atmosphere packaging and low-dose irradiation on toxin production by *Clostridium botulinum* in fresh pork. *J. Food Protect.* **54**:94-101.

Lambert, R. (1990). Value-added shrimp products in Europe. *Infofish Int.* **4**:11-14.

Lanier, T.C. and Thomas, F.B. (1978). *Minced Fish: Its Production and Use.* North Carolina State University Sea Grant College Program Publications, North Carolina, U.S.A.

Larsen, H.E. and Gundstrup, A. (1965). The inhibitory action of various strains of *Bacillus* on *Listeria monocytogenes. Nord. Vet. Med.* **17**:336-341.

Laycock, R.A. and Regier, L.W. (1971). Trimethylamine-producing bacteria on haddock (*Melanogrammus aeglefinus*) fillets during refrigerated storage. *J. Fish. Res. Board Can.* **28**:305-309.

Lee, C.M. (1984). Surimi process technology. *Food Technol.* **38**:69-80.

Lee, C.M. (1986). Surimi manufacturing and fabrication of surimi-based products. *Food*

Technol. **40**:115-124.

Lennon, D., Lewis, B., Mantell, C., Becroft, D., Dove, B., Farmer, K., Tonkin, S., Yeates, N., Stamp, R. and Mickleson, K. (1984). Epidemic perinatal listeriosis. *Pediatr. Infect. Dis.* **3**:30-34.

Lerke, P., Adams, R. and Farber, L. (1965). Bacteriology of spoilage of fish muscle, part-III: characterization of spoilers. *Appl. Microbiol.* **13**:625-630.

Liston, J. (1980). Microbiology of seafoods. In: Connell, J.J. (ed.) *Adv. Fish Sci. & Technol.* Fishing News (Books), Surrey, U.K. pp. 138-157.

Lovett, J. (1989). *Listeria monocytogenes*. In: Doyle, M.P. (ed) *Foodborne bacterial Pathogens*. Marcel Dekker Inc., New York, U.S.A. pp. 283-310.

Lucke, F.K. and Roberts, T.A. (1993). Control in meat and meat products. In: Hauschild, A.H.W. and Dodds, K.L. (eds.) *Clostridium botulinum: Ecology and Control in Foods.* Marcel Dekker Inc., New York, U.S.A. pp. 177-207.

Lyver, A. (1997). *M. Sc. Thesis: Formulation, shelf-life and safety studies on value-added seafood products*. McGill University, Quebec, Canada.

Lyver, A., Smith, J.P., Nattress, F.M., Austin, J.W. and Blanchfield, B. (1998). Challenge studies with *Clostridium botulinum* type E in a value-added surimi product stored under a modified atmosphere. *J. Food Safety.* **18**:1-23.

Marshall, D.L., Andrews, L.S., Wells, J.H. and Farr, A.J. (1992). Influence of modified atmosphere packaging on the competitive growth of *Listeria monocytogenes* and *Pseudomonas fluorescens* on precooked chicken. *Food Microbiol.* **9**:303-309.

Marshall, D.L., Wiese-Lehigh, P.L., Wells, J.H. and Farr, A.J. (1991). Comparative growth of *Listeria monocytogenes* and *Pseudomonas fluorescens* on precooked chicken nuggets stored under modified atmospheres. *J. Food Protect.* **54**:841-843.

Martin, R.E. (1976). Mechanically-deboned fish flesh. *Food Technol.* **30**:64-70.

Matches, J.R., Liston, J. and Curran, D. (1974). *Clostridium perfringens* in the environment. *Appl. Microbiol.* **28**:655-660.

McAdams, T.J. (1996). *M. Sc. Thesis: The determination of microbial quality and the presence of pathogens and chemical contaminations in aquacultured rainbow trout (Onchorhyncus mykiss) fillets and whole fish from different aquacultured production systems*. Virginia Polytechnic Institute and State University, Virginia, U.S.A.

McCarthy, S.A. (1997). Incidence and survival of *Listeria monocytogenes* in ready-to-eat seafood products. *J. Food Protect.* **60**:372-376.

McClure, P.J., Cole, M.B. and Smelt, J.P.P.M. (1994). Effects of water activity and pH on growth of *Clostridium botulinum*. *J. Appl. Bacteriol. Symposium Supplement.* **76**:105-114S.

McClure, P.J., Kelly, T.M. and Roberts, T.A. (1991). The effects of temperature, pH, sodium chloride, and sodium mitrite on the growth of *Listeria monocytogenes*. *Int. J. Food Microbiol.* **14**:77-92.

Mclaughlin, J., Audurier, A. and Taylor, A.G. (1986). The evaluation of a phage-typing system for *Listeria monocytogenes* for use in epidemiological studies. *J. Med. Microbiol.* **22**:357-365.

Meyer, K.F. and Dubovsky, B.J. (1922). The distribution of the spores of *Clostridium botulinus* in the United States, IV. *J. Infec. Disease.* **31**:559-594.

Miettinen, M.K., Silronen, A., Heiskanen, P., Haajanen, H., Bjorkroth, K.J., and Korkeala, H.J. (1999). Molecular epidemiology of an outbreak of febrile gastroenteritis caused by Listeria monocytogenes in cold-smoked rainbow trout. *J. Clin. Microbiol.* **37**:2358-2360.

Mitsuda, H.K., Nakajima, K., Mizuno, H. and Kawai, F. (1980). Use of sodium chloride for extending the shelf life of fish fillets. *J. Food Sci.* **45**:661-666.

Miyake, Y., Hirasawa, Y. and Miyanabe, M. (1985). Technology of surimi manufacturing. *Infofish.* 5/85:29-32.

Morris, J.E. (1995). *M. Sc. Thesis: The combined effect of modified atmosphere packaging (MAP) and chitosan on the growth of Listeria monocytogenes in model systems and in fresh pork loin.* McGill University, Quebec, Canada

Naganawa, R., Iwata, N., Ishikawa, K., Fukuda, H., Fujino, T. and Suzuki, A. (1996). Inhibition of microbial growth by ajoene, a sulfur-containing compound derived from garlic. *Appl. Env. Microbiol.* **62**:4238-4242.

Newton, K.G. and Rigg, W.J. (1979). The effect of film permeability on the storage life and microbiology of vacuum-packed meat. *J. Appl. Bacteriol.* **47**:433-441.

Nickelson, R., Finne, G., Hanna, M.O. and Vanderzant, C. (1980). Minced fish flesh from nontraditional Gulf of Mexico finfish species: bacteriology. *J. Food Sci.* **45**:1321-1326.

Nickerson, J.T.R., Goldblith, S.A., Digioia, G. and Bishop, W.W. (1967). The presence of *Clostridium botulinum* type E in fish and mud taken from the Gulf of Maine. In: Ingram M.

and Roberts T.A. (eds.) *Botulism 1966.* Chapman and Hall, London, U.K. pp. 25-33.

Nielsen, J.W., Dickson, J.S. and Crouse, J.D. (1990). Use of a bacteriocin produced by *Pediococcus acidilactici* to inhibit *Listeria monocytogenes* associated with fresh meat. *Appl. Env. Microbiol.* **56**:2142-2145.

Nieman, R.E. and Lorber, B. (1980). Report of eight cases and review of the literature, 1968-78 – Listerosis in adults: a changing patterns. *Rev. Infec. Disease.* **2**:207-227.

Niemand, K.G., van der Linde, H.J. and Smith, K.M. (1981). Radurization of prime beef cuts. *J. Food Protect.* **44**:53-59.

Ogrydziak, D.M. and Brown, W.D. (1982). Temperature effects in modified-atmosphere storage of seafoods. *Food Technol.* **36**:5.

Oh, D.H. and Marshall, D.L. (1994). Enhanced inhibition of *Listeria monocytogenes* by glycerol monolaurate with organic acids. *J. Food Sci.* **59**:1258-1261.

Okereke, A. and Montville, T.J. (1991a). Bacteriocin inhibition of *Clostridium botulinum* spores by lactic acid bacteria. *J. Food Protect.* **54**:349-353.

Okereke, A. and Montville, T.J. (1991b). Bacteriocin-mediated inhibition of *Clostridium botulinum* spores by lactic acid bacteria at refrigeration and abuse temperatures. *Appl. Env. Microbiol.* **57**:3423-3428.

Okrend, A.J.G., Rose, B.E. and Bennett, B. (1987). Incidence and toxigenicity of *Aeromonas* species in retail poultry, beef and pork. *J. Food Protect.* **50**:509-513.

Palumbo, S.A., Morgan, D.R. and Buchanan, R.L. (1985). Influence of temperature, NaCl, and pH on the growth of *Aeromonas hydrophila. J. Food Sci.* **50**:1417-1421.

Parish, M.E. and Higgins, D.P. (1989). Survival of *Listeria monocytogenes* in low pH model broth systems. *J. Food Protect.* **52**:144-147.

Peltroy, G.A., Eklund, M.W., Parajpye, R.N., Suzuki, E.M. and Peterson, M.E. (1982). Inhibition of *Clostridium botulinum* type A and E toxin formation by sodium nitrite and sodium chloride in hot-processed (smoked) salmon. *J. Food Protect.* **45**:833-841.

Pilet, M.F., Dousset, X., Barre, R., Novel, G., Desmazeaud, M. and Piard, J-C. (1995). Evidence for two bacteriocins produced by *Carnobacterium piscicola* and *Carnobacterium divergens* isolated from fish and active against *Listeria monocytogenes. J. Food Protect.* **58**:256-262.

Pigott, G.M. (1986). Surimi: the "high tech" raw materials from minced fish flesh. *Food Rev. Int.* **2**:213-246.

Powers, E.M., Thomas, G.L. and Brown, T. (1976). Incidence and levels of *Bacillus cereus* in processed spices. *J. Milk food Technol.* **39**:668-670.

Price, R.J. and Lee, J.S. (1970). Inhibition of *Pseudomonas* species by hydrogen peroxide producing lactobacilli. *J. Milk Food Technol.* **33**:13-18.

Punyasingh, T. (1992). *ThaiLife: Thai Cuisine*. The National Identity Board, Bangkok, Thailand. p. 59.

Quinn, E.L, and Jones, C.L. (1936). *Carbon Dioxide*. Reinhold, New York, U.S.A.

Razavilar, V. and Genigeorgis, C. (1992). Interactive effect of temperature, atmosphere, and storage time on the probability of colony formation on blood agar by four *Listeria* species. *J. Food Protect.* **55**:88-92.

Regenstein, J.M. (1986). The potential for minced fish. *Food Technol.* **40**:101-106.

Roessink, G.L. (1989). Battered and breaded products. *Infofish Int.* **4**:17-20.

Ryser, E.T. and Marth, E.H. (1991). *Listeria, Listeriosis, and Food Safety.* Marcel Dekker Inc., New York, U.S.A.

Sakaguchi, G. (1979). Botulism. In: Reimann, H. (ed) *Food-borne Infections and Intoxications*. Academic Press, New York, U.S.A. pp. 390-433.

Schilinger, U., Kaya, M. and Lucke, F-K. (1991). Behavior of *Listeria monocytogenes* in meat and its control by bacteriocin-producing strain of *Lactobacillus sake*. *J. Appl. Bacteriol.* **70**:473-478.

Schmidt, C.F., Lechowich, R.V. and Folinzao, J.J. (1961). Growth and toxin production by type E *Clostridium botulinum* below 40°F. *J. Food Sci.* **26**:626-630.

Sears, D.F. and Eisenberg, R.M. (1961). A model representing a physiological role of carbon dioxide at the cell membrane. *J. Gen. Physiol.* **44**:869-887.

Seeliger, H.P.R. (1961). *A Listeriosis.* Hafner, New York, U.S.A.

Shewan, J.M., Hobbs, G. and Hodgkiss, W. (1960). The *Pseudomonas* and *Achromobacter* groups of bacteria in the spoilage of marine white fish. *J. Appl. Bacteriol.* **23**:463-468.

Sheridan, J.J., Doherty, A., Allen, P., McDowell, D.A., Blair, I.S. and Harrington, D. (1995). Investigations on the growth of Listeria monocytogenes on lamb packaged under modified atmospheres. *Food Microbiol.* **12**:259-266.

Shineman, T.L. and Harrison, M.A. (1994). Growth of *Listeria monocytogenes* on different muscle tissues. *J. Food Protect.* **57**:1057-1062.

Simpson, L.L. (1986). Molecular pharmacology of botulinum toxin and tetanus toxin. *Annual Rev. Pharmac. & Toxic.* **26**:427.

Smikahl, J.R. (1985). *M. Sc. Thesis: Action of carbon dioxide on myoglobin and mitochondrial enzymes in beef heart extracts.* University of California-Davis, California, U.S.A.

Smith, J.P. (1993). Bakery products. In: Parry, R.T. (ed.) *Principles and Applications of Modified Atmosphere Packaging of Food.* Blackie Academic and Professional, London, U.K. pp. 134-169.

Smith, J.P. (1994). Modified atmosphere packaging for bakery products. *Research Department Technical Bulletin, Vol. XVI, March.* Issue 3.

Smith, J.P., Simpson, B.K. and Ramaswamy, H. (1990a). Developments in food packaging technology: part I – process and cooking consideration. *Trends Food Sci. Technol.* **1**:107-111.

Smith, J.P., Simpson, B.K. and Ramaswamy, H. (1990b). Developments in food packaging technology: part II – storage aspects. *Trends Food Sci. Technol.* **1**:112-119.

Smith, L. and Sugiyama, H. (1988). *Botulism: the organism, its toxins, the disease*. Charles C. Thomas, Illinois, U.S.A.

Smith, L.D.S. (1975). Inhibition of *Clostridium botulinum* by strains of *Clostridium perfringens* isolated from soil. *Appl. Microbiol.* **30**:319.

Smith, R., Nickerson, R., Martin, R and Finne G. (1984). Bacteriology of indole production in shrimp homogenates held at different temperatures. *J. Food Protect.* **47**:861-864.

Sofos, J.N., Busta, F.F. and Allen, C.E. (1979). Botulism control by nitrite and sorbate in cured meats: a review. *J. Food Protect.* **53**:831-833.

Sonu, S. (1986). Surimi. *National Oceanic and Atmospheric Administration Technical Memorandum.* NMFS-SWR-013.

Sperber, W.H. (1977). The identification of staphylococci in clinical and food microbiology laboratories. *CRC Crit. Rev. Clin. Lab. Sci.* 7:121-184.

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

Sugiyama, H. and Yang, K.H. (1975). Growth potential of *Clostridium botulinum* in fresh mushrooms packaged in semipermeable plastic film. *Appl. Microbiol.* **30**:964-969.

Tanaka, N., Meske, L., Doyle, M.P., Traisman, E., Thayer, D.W. and Johnson, R.W. (1985). Plant trials of bacon made with lactic acid bacteria, sucrose, and lowered sodium nitrite. *J. Food Protect.* **48**:679-686.

Tanaka, N., Traisman, E., Lee, M.H., Cassens, R.G. and Foster, E.M. (1980). Inhibition of botulinum toxin formation in bacon by acid development. *J. Food Protect.* **43**:450-457.

Taylor, L., Cann, D.D. and Welch, B.J. (1990). Antibotulinal properties of nisin in fresh fish packaged in an atmosphere of carbon dioxide. *J. Food Protect.* **53**:953-959.

TIME-LIFE Books. (1979). *The Good Cook Techniques & Recipes: Fish.* TIME-LIFE Books, Virgina, U.S.A. p. 149.

Venugopal, V. and Shahidi, F. (1994). Thermostable water dispersions of myofibrillar proteins from Atlantic mackerel (*Somber scombrus*). *J. Food Sci.* **59**:265-268.

Ward, D. R. (1989). Microbiology of aquaculture products. *Food Technol.* **43**(11):82-87.

Watts, D.A. and Brown, W.D. (1982). Histamine formation in abusively stored Pacific mackerel: effect of CO_2 modified atmosphere. *J. Food Sci.* **47**:1386-1387.

Weagant, S.D., Sado, P.N., Colburn, K.G., Torkelson, J.D., Tanley, F.A., Krane, M.H., Shields, S.C. and Thayer, C.F. (1988). The incidence of *Listeria* species in frozen seafood products. *J. Food Protect.* **51**:655-657.

Wentz, M.W., Scott, R.A. and Vennes, J.W. (1967). *Clostridium botulinum* type F: seasonal inhibition by *B. licheniformis*. *Science.* **155**:89-90.

Wuthe, H.H. and Findel, G. (1972). *Salmonellae* in flat fishes from coastal waters. *Arch. Lebens. Hyg.* **23**:110-111.

Yasuda, M., Nishino, H., Tanaka, M., Chiba, T., Nakano, H., Yodoyama, M. and Ogawa, S. (1992). Studies on gas-exchange packaging of fresh fish, part 2: preservation of freshness of rainbow trout fillets packaged with carbon dioxide-nitrogen gas mixture. *Packaging*

Technol. Sci. **5**:109-113.

Yoon, I.H., Matches, J.R. and Rasco, B. (1988). Microbiology and chemical changes of surimi-based imitation crab during storage. *J. Food Sci.* **53**:1343-1346.

Young, L.L., Revere, R.D. and Cole, A.B. (1988). Fresh red meats: a place to apply modified atmosphere. *Food Technol.* **42**(9):65-9.