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Review:

Food Additives and their use in Destruction of Some Seafood Poisoning Bacteria

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Abstract:

The seafood industry is always searching for new preservation strategies to extend the shelf life of fish and provide the consumers with fish material with the best quality. Decontamination of Fish products using organic acids and other chemical treatments has been and continues to be one of the most important interventions for controlling their microbiological safety and quality. This chapter first covers

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aspects pertinent to the principles and technology of decontamination with chemical agents, and then reviews, food decontamination applications of chemical treatments, with a particular emphasis on organic acids, as well as information regarding their mode of action and effectiveness against spoilage and/or pathogenic bacteria. Additional topics discussed in the chapter include the potential effects of chemical decontamination on food quality, concerns and risks other than food quality associated with this type of intervention, and regulatory aspects of its implementation. The quality and freshness of marine species rapidly decline postmortem due to a variety of microbial and biochemical degradation mechanisms. Modified atmosphere packaging (MAP) is an increasingly popular food preservation technique. Consumer demands for fresh and convenient foods free of chemical preservation has led to expanding the use of MAP and this technique may also reduce wastage and extends shelf-life of a range of seafood.

Key words: Fish decontamination, microbiological safety and quality, organic acids, Modified Atmospheric Packaging.

Introduction

Fish and shellfish are excellent protein sources for human consumption. In addition, they have higher contents of hydrosoluble and lipsoluble vitamins, minerals and polyunsaturated fatty acids (PUFAs). Interestingly, omega-3 fatty acids, found mainly in fat-rich fish such as salmon, mackerel, herring, and sardines confer health benefits to humans not found in any other foods. Omega-3 fatty acids from fish can lower blood triglycerides, reduce abnormal heart rhythms, reduce blood pressure by small but significant amounts and improve blood clotting regulation (Nettleton, 1995). There are inherent limitations with the convention of grouping fatty acids based only on the number of double bonds, i.e. saturated fatty acids (SFA), monounsaturated fatty acids

(MUFA) and polyunsaturated fatty acids (PUFA) insofar as describing the effects of fatty acids in human health and in developing dietary recommendations. The large body of epidemiological evidence about total fats, fatty acids, and human health apply these groupings and show that the major groups of fatty acids are associated with different health effects (Nettleton, 1991). Fish is very important in the human diet because it supplies a high nutritional value protein along with a variety of vitamins and minerals (Jiang et al, 2008).

Fish spoilage and foodborne pathogenic bacteria

Marine fish products deteriorate rapidly post mortem as a consequence of various microbial and biochemical breakdown processes. The rate of quality loss depends directly on the nature of the fish species in question, as well as the handling and storage conditions. The quality of wild caught cod may vary considerably, due to seasonal variations, different handling, fishing gear and fishing ground. The time between the catch and processing will in addition strongly influence the quality. Compared to wild caught fish, farmed fish have several advantages as a raw material. Wild caught cod is known to have a different body composition than farmed cod, with a higher condition factor, smaller head and larger liver (Gildberg, 2004). There is also observed a higher carbohydrate level and lower pH in the muscle (Rustad, 1992). Shelf life studies on the MA-packaged wild fish have shown the importance of temperature, production hygiene and gas composition on the development of specific spoilage organisms (SSO) (Gram and Dalgaard, 2002). The bacterial flora of wild cod stored aerobically on ice is well studied and dominated by *Pseudomonas sp*. (Gram and Dalgaard, 2002), *Shewanella baltica, Shewanella hafniensis and Shewanella morhuae* (Vogel et al., 2005; Satomi et al, 2006), rather than *Shewanella*

putrefaciens, which has previously been considered, in many studies, as the main spoilage organism (Gram and Dalgaard, 2002). In a modified atmosphere (MA) packaging with high CO_2 concentration, the CO_2 tolerant bacterium Photobacterium phosphorus has been identified as the main organism responsible for spoilage (Corbo *et al.,* 2009). Knowledge about spoilage organisms and their specific activity in various fish species at different storage conditions has led to more precise shelf life predictions and facilitated modeling of spoilage (Gram and Dalgaard, 2002). Thus, in order to develop new high quality products it is important to have rapid and precise methods to analyze changes in the microbial community as a function of hygienic handling, packaging and storage. Publications during the last decade tend to present an investigation of the whole microbial community, rather than sub populations, by using culture independent methods (Corbo *et al.,* 2009).. Recently the US Food and Drug Administration (FDA, 2001) issued a series of consumption advisories based on methylmercury that suggested that pregnant women and women of childbearing age who may become pregnant should avoid eating four types of marine fish, shark, swordfish, king mackerel, and tilefish, and should limit their consumption of all other fish to just 12 ounces per week (FDA, 2001). These recent FDA (2001) advisories have raised concern about the safety of fish available in supermarkets, yet there are very few data on contaminant levels in commonly available commercial fish. Data from FAO (Anonymous, 1999) show that Spain is among the top 14 countries ranked by the total production of aquaculture products. Available information (Anonymous, 1999) shows that aquaculture products, 314,000 tonnes in total, constitute 24% of the total Spanish fishery products, with freshwater fish accounting for ca. 10% of this figure. The presence of human pathogenic bacteria in aquaculture products is dependent on a number of factors, including method of production, rearing

practices, environmental conditions and the methods use to harvest, process and distribute the products. (Arvanitoyannis, Ioannis, 2009). Food poisoning organisms in fish are often divided into two groups: those that are naturally present in freshwater environments, referred to as indigenous bacteria, and those associated with pollution of the aquatic environment.

A third group includes bacteria introduced to fish and fish products during post-harvest handling and processing (Arvanitoyannis, Ioannis, 2009). Three indigenous bacteria of recent interest are *Edwarsiella tarda, Plesiomonas shigelloides* and motile *aeromonads* (Arvanitoyannis, Ioannis, 2009).

Listeria monocytogenes

L. monocytogenes was first described in 1924 by (Murray et al., 1926). The organism was isolated from rabbits and guinea pigs and observed to cause *monocytogenes* in the infected animals. The bacterium was originally named Bacterium *monocytogenes* (Nyfeldt, 1992). *Listeria monocytogenes* is a Gram-positive, foodborne pathogen. It is widely distributed in the environment and occurs naturally in many raw foods. *Listeria monocytogenes* is *psychotropic* and halotolerant and can, under otherwise optimal conditions, grow in the range of 1 to 45 °C (34 to 113 °F) and between 0 and 10% NaCl. As a consequence, it may grow in many food products with extended shelf lives (Rørvik and Yndestad, 1991). *Listeria monocytogenes* can grow in a wider temperature range, from -1.5 to 45°C (Hudson et al., 1994). It has already long been established as an important food borne pathogen. However, the incidence in food related listeriosis outbreaks has increased dramatically in the last few years and *L. Monocytogenes* is now considered a pathogen of major concern. A diverse range of food has been associated with outbreaks of listeriosis (McLauchlin, 1997). Various food products are consumed without further cooking and are also

capsule of supporting growth of *L. monocytogenes*. A range of seafoods, particularly the lightly preserved products (6% water-phase salt, pH 5) such as smoked fish products, lightly salted products (e.g. Brined cooked shrimp) or marinated products fall within this category. Outbreaks of listeriosis associated with smoked mussels, smoked trout and raw oysters, have been reported (Duy et al., 2000) Therefore, some degree of poisoning risk from seafood is evident.

Prevalence of *Listeria monocytogenes* in raw fish

L. monocytogenes is an organism indigenous to the general environment and occurs in the gastrointestinal tract of 2-6% of healthy humans (carriers). It is not typical of aquatic and marine environments. Thus the organism cannot be isolated from free, open waters nor from fish caught or cultured in such waters in contrast, water close to agricultural run-off harbors the pathogen and it is assumed to be present, albeit at low levels on raw fish (Huss *et al.,* 2000). *L. monocytogenes* could easily be isolated from processed fish products. Three to fourteen percent of ready- to- eat (RTE) seafood are positive for *L. Monocytogenes* but in some smoke houses as many as 80% of the samples are positive. The pathogen is isolated at much higher frequency from RTE seafood products than from raw materials. Several studies have demonstrated that the processing environment is an important niche for *L. monocytogenes* (Autio *et al.,* 1999). It is also detected in heat-processed products subjected to a listericidal process. Post-process contamination is the likely cause of this contamination. Cleaning and disinfection may temporarily remove the organism which is often found in most permanent niches in drains or floor mats. Products that do not receive a heat treatment by the consumer, including ready-to eat (RTE) products such as cheeses, meat, and fish delicatessen products, may contain high levels of *L. monocytogenes* when eaten, and

many of these types of foods have been associated with listeriosis (McLauchlin 1997). In general, populations in foods are low (log 0 to 10^3 cfu/g with 90 to 99% being below log 10^2 cfu/g and less than 1% being between log 10^3 and 10^4 cfu/g) (Jørgensen and Huss 1998) however, higher concentrations (10^5 to 10^7 cfu/g) have been reported (Farber, 1991).

Growth and survival of fish and fishery products

Listeria monocytogenes is halo- and psychrotolerant and can grow well in refrigerated foods. It is difficult to control it in RTE seafood products where there is no listericidal processing step and where *L. monocytogenes* can grow at the temperature / ^{a}w / atmosphere conditions prevailing in the products. Several studies have demonstrated that it grows (rapidly) in brined shrimp and cold smoked fish. This may partly be explained by the so-called Jameson effect where the presence of a competitive associate micro flora depresses the maximum cell density of the bacterium. The NaCl concentration is critical when evaluating growth potential, as the bacterium may grow rapidly at 3-4% NaCl but much slower with 7-8% NaCl. *L. monocytogenes* is of little importance in semi-preserved seafood products were 2.5% acetic acid is used. Also, use of citric acid can be used to clean floors and drains and eliminate the organism from processing environments. Nitrate, lactate, di-acetate and bacteriocins inhibit or delay growth. Protective competitive lactic acid bacterial flora that inhibit *L. monocytogenes* (Nilsson *et al.,* 2000).

Prevalence in water, raw fish

Listeria monocytogenes is a ubiquitous organism (Farber, 1991). Although its natural niche is probably soil and Vegetation, it can readily be isolated from fresh and marine waters. (Ben Embarek, 1994) reviewed a number of studies and found that the prevalence in a variety of water bodies (river waters,

seawater, surface water, spring water) varied from 0 to 62%. Notably, the highest number of positive samples was found in waters exposed to runoff from agricultural or urban areas, whereas waters such as spring water or free ocean waters were negative for the organism. (Fenlon and others 1996) investigated river waters in the United Kingdom and found 17 of 36 samples to be positive for *L. monocytogenes*, with levels in positive samples ranging from log 10 to 350 cfu/liter. (Farber,1991) reported the presence of *L. monocytogenes* in salmon from the United States, Chile, Norway, and Canada. The prevalence of the pathogen in raw fish is low, ranging from 0–1% to 10% (Jemmi and Keusch 1994). Only 1.3% of 781 samples of Japanese fish contained *L. monocytogenes* (Iida *et al.* 1998), and none of 60 raw salmon sampled in Japan were positive for the bacterium (Jemmi, 1993). The literature reviewed by (Ben Embarek, 1994) indicated large variations, with 0 to 50% of fresh fish samples positive. Unpublished information indicates that the prevalence in fish in mud freshwater ponds or fish in seawater nets close to land runoff can be as high as 100%. It could be speculated that fish reared in waters close to agricultural runoff would carry a higher load of *L. monocytogenes* than fish cultured in waters free from such soil and vegetation sources.

Prevention and control

Control of *L. monocytogenes* can be using sufficient levels of acid and NaCl will also prevent growth. Sorbate (0.05-0.1%) or the combination of lactate (2%) and di-acetate (0.1%) has been shown to eliminate growth in frankfurters (Tompkin, 1999). As mentioned, the addition of live lactic acid bacteria may inhibit growth in some products. Frozen storage is an efficient way to completely prevent the growth of *L. monocytogenes*. If the product is vacuum-packaged, frozen storage will have few adverse effects on sensory quality (that is, lipid oxidation). One

study has reported growth of *L. monocytogenes* at –1.5 °C (29 °F) in roast beef, but the generation time at this temperature was 129 he and other studies have found the minimum temperature for growth in 0.1°C to 1.1 °C (32 to 34 °F) (Farber ,1991). Refrigeration and vacuum storage do not guarantee prevention of *L. monocytogenes* growth. *L. monocytogenes* is able to grow from log10^5 to 10^9 cfu/g at 12°C and from log 10^4 to 10^9 or 10^5 cfu/g at 6 and 0°C, respectively, on crawfish tail suggesting that chilled temperature storage is not sufficient to prevent *L. monocytogenes* growth. These authors also showed that freezing of the finished product did not kill *L. monocytogenes* and did not affect its development later.

Escherichia coli

E. coli O157:H7 is a member of the enterohemorrhagic group of pathogenic *E. coli* that has emerged as a foodborne and waterborne pathogen of major public health concern. A wide variety of foods have been implicated as vehicles of *E. coli* O157:H7 infection, including meat, milk, fruit juices, and vegetables (Buchanan and Doyle, 1997). Unlike most foodborne pathogens, *E. coli* O157:H7 is tolerant of acidic environments. Survival in apple cider (pH 3.6–4.0) and mayonnaise (pH 3.6–3.9) has been reported and *E. coli* O157:H7 survived fermentation of buttermilk (pH 4.4) and drying and storage of fermented sausage (pH 4.5) (Buchanan and Doyle, 1997). These organisms cause a spectrum of disease increasing in severity from a mild diarrheal illness to hemorrhagic colitis, hemolytic uremic syndrome, and, in some cases, death (Boles *et al.,* 1993). Several years ago, hurdle technology was developed as a new concept for the realization of safe, stable, nutritious, tasty, and economical foods. This approach uses a combination of suboptimal growth factors, e.g. heating, chilling, drying, salting, conserving, acidification, oxygen-removal, fermenting,

adding various preservatives, to establish growth inhibition of microorganisms in foods (McMeekin *et al.,* 2000). *Escherichia* is a member of the *Enterobacteriaceae* family and is the most common organism in the intestinal tract of man and warm-blooded animals. Most of the *E. coli* strains are harmless. *E. coli* colonizes the intestinal tract and probably plays important roles in maintaining intestinal physiology. However, some strains of *E. coli* are pathogenic and can cause diarrhoeal disease. *E. coli* strains are differentiated based on a serotyping scheme involving O (somatic), H (flagellar) and K (capsular) antigens. Pathogenic *E. coli* are divided into specific groups depending on virulence, clinical symptoms and distinct O: H antigens (Davidson and Juneja 1990).

Prevalence in fish and fishery products

The main source of *E. coli* infections have been (faecally) contaminated water and food handlers. Outbreaks by enteroheamorraghic (EHEC) have mostly involved undercooked ground beef and raw milk. Also vegetables, such as alfalfa sprouts, washed or cultured in contaminated water have caused outbreaks. An unusual number of outbreaks have been related to unpasteurized apple juice in the USA. Due to the relatively low pH, these juices were considered safe; however EHEC strains have an unusual acid tolerance and thus survived in the product. Neither of the *E. coli* strains are typical of aquatic products. However, poor hygiene, cross contamination by food handlers or dirty water may transfer the organism. Also, such strains may accumulate in filter feeding bivalves cultured in contaminated waters. While *E. coli* is not indigenous to the aquatic environment, it may survive and even multiply in warm tropical waters (Rehbein and Oehlenschlager, 1996).

Growth and survival in fish and fishery products

E. coli strains are mesophilic organisms with optimum growth at 37°C. They do not grow at chill temperatures and are readily destroyed by mild heating. Most isolation procedures rely on incubation at 44°C, However, EHEC strains do not grow on selective media at 44°C. In general, the organisms are sensitive to salt and acidifying (Nanni, 1998).

Additives used in fish preservation

Fish spoilage and foodborne pathogenic bacteria

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were marketed in Spanish supermarkets and multiple chain stores (Rodriguez-Garcia and Guil-Guerrero, 2008). The presence of human pathogenic bacteria in aquaculture products is dependent on a number of factors including method of production, rearing practices, environmental conditions and the methods use to harvest, process and distribute the products (Gram *et al,*.2002). Food poisoning organisms in fish are often divided into two groups: those that are naturally present in freshwater environments, referred to as indigenous bacteria, and those associated with pollution of the aquatic environment. A third group includes bacteria introduced to fish and fish products during post-harvest handling and processing (ICMSF, 1998). Three indigenous bacteria of recent interest are *Edwarsiella tarda, Plesiomonas shigelloides* and motile *aeromonads* (Gram *et al,*.2002).. Enteric organisms as *Salmonella* can enter aquaculture systems from many sources, including farm runoff and direct contamination from wild animals, livestock and feed. Poor hygienic practices after harvesting may also contaminate aquaculture products. *Listeria* species, that do not appear to have a natural reservoir in fish, are considered nonindigenous bacteria of the third group (Kvenberg, 1991). Spoilage of fish and crustaceans takes place as soon as seafood species die. The natural defense mechanisms, then stop functioning and a series of changes in seafood start and thus begin that causing spoilage of fish (dead). Bacteria, enzymes and chemical action cause these changes. Bacteria are the most important cause of seafood spoilage. Bacteria are present in large number in surface slime, gills, and in the intestines of living seafood. When a fish dies, bacteria, or their enzymes invade the flesh through the gills, along blood vessels, and skin and other parts of the fish. Then the bacteria grow and multiply in flesh producing various compounds responsible for fishy odors and discolorations muscles. High temperatures speed spoilage and low

temperatures slow spoilage (Gram, 1996). Sanitation is also important. Contamination of seafood by bacteria from dirty ice, containers and surfaces can increase the number of bacteria on seafood and speed spoilage. Contamination with food poisoning bacteria can cause illness when the seafood is eaten. Keeping seafood handling and storage equipment clean, reduces bacteria contamination and slows spoilage. (Tzikas *et al*., 2007) reported that the flesh of live healthy fish is sterile; microorganisms (the natural bacteria flora) are only present on the outer surface (gills, skin) and in the gastrointestinal tract. After death a complex series of enzyme changes take place in the fish muscle and the flesh remains sterile or nearly so for 3-4 days at 0°C (Shewan, 1962).seafood highly susceptible to spoilage and deterioration because of analysis and growth of microbial populations (Jeyasekaran *et a.,* 2006). Four factors which influence the kind and rate of fish spoilage. These were the kind of fish, the condition of the fish when caught, the kind and extent of contamination of the fish flesh with bacteria, and temperature. (Gennari *et al.,*1988) found that *pseudomonas* SPP. was the predominant bacteria genera in thawed Mackerel at the time of spoilage. In addition, (Papadopoulos *et al.,* 2003) found that the *Shewanella putrefactiens* and *pseudomonas* SPP. Were the specific spoilage bacteria in fish from temperate and tropical waters and in fresh Mediterranean fish stored aerobically under refrigeration or in ice. (Ababouch *et al.,* 1996) suggested that the proliferation of non-H_2s producing bacteria such as *pseudomonas* can contribute significantly to the spoilage of sardines caught in the Atlantic Ocean. The spoilage of fresh fish has been found to be a rather complex process. There is no one factor or system, which is solely responsible for it. Rather, a number of interrelated systems, some of which are suppressed by others, cause spoilage. Among those factors which principally contribute to spoilage are: the degradation of nucleotides with subsequent forming of hypoxanthine (Hx)

formation trimethyline (TMA); development of oxidative rancidity; and the action of certain bacteria (Martin *et al.,* 1993). (Mahmoud *et al.,* 2004) showed that the spoilage of fresh water fish occurs within 5-8 days under refrigerated temperatures. Bacterial spoilage in refrigerated fish and fish products under aerobic storage conditions is caused by Gram-negative *psychotrophic* organisms such as *pseudomonas, Alteromonas, shewanella* and *Falfobacterium* spp. The dominant microorganisms involved in the decomposition/ spoilage of shrimp are psychrotrophic bacteria, such as pseudomonas *and Moraxella/ Acinetobacter species* (Ward and Hackney, 1991). Temperature influences the rate of food spoilage by its effects on both enzymatic and microbial activity. The growth of many microorganisms is reduced at temperatures below 10°C and even cold-tolerant bacteria have much longer phase and generation times at temperature near 0°C (Huss, 2000). The most effective method of preserving fresh fish is by chilling to about 0-1°C (Pedersoa-Menabrito and Regenstein, 2000). On December 18, 1995 the long anticipated regulation for a new method of inspection for food safety in the seafood and aquaculture industry was adopted by the U. S. Food & Drug Administration (FDA). The new system was named the HACCP, Hazard Analysis and Critical Control point programs designed to prevent and control food safety problems. The justification for the program was based on continuing concerns for seafood-borne illnesses, public expectations, industry requests, and market trends in both domestic and international settings (Center for food safety and Applied Nutrition, 2002).

Definition of food additives

Chemical and natural substance added to food during preparation or achieving a particular a part of the food or

affects its characteristics for the purpose of appearance, texture, or keeping quality of a food or serve as essential aids in the processing of food are all considered to be food additives. Organic acids have been used for decades in food preservation, protecting feed from microbial and fungal hazards. In particular acetic acid, citric acid and lactic acid have been used with meats extensively for a positive influence on pathogen bacteria such as *L. monocytogenes* and *E. coli* and other spoilage (Jiang, and Zhou, 2003). Acetic acid, citric acid, and lactic acid have been reported to effectively reduce large populations of *E. coli* O157:H7 and Salmonella (Torrieri *et al.,* 2006). Organic acids are the primary metabolites found in great amounts in all plants, especially in fruits. Citric, malic and tartaric acids are commonly found in fruits and berries, while oxalic acid is present in higher amounts in green leaves. As phenolics, the organic acids may also have a protective role against bacteria, diseases due to their antioxidant properties (Beuchat, 2001). Chemical preservative substances are two parts; inorganic preservatives (e.g. NO_2 and organic preservatives such as lactic, citric, acetic, benzoic and propionic. Ascorbic acids and other acids ware used for fish, pork, poultry preservation (Alasia *et al.* 1991). In general, the preservative action of these compounds is mainly due to their inhibitory effect not only on the metabolism of microorganism but also on their growth. None of these chemicals have a complete spectrum of action against the entire spoilage microorganism likely to occur in fish and fish products. With most of the preservatives, the predominant action is against Yeast and Molds (Frazier and Westhoff, 1978). Many physical or chemical treatments have been used to eliminate or inhibit the growth of microorganisms in foods. Food processing often involves the use of acidulated additives, refrigeration, heating or freezing to control microbial proliferation. Fish among other fresh foods which are considered susceptible foodstuff to different types of

spoilage such as analysis, oxidation, hydrolysis of fats as well as microbial spoilage. Therefore, different methods are used for delaying fish spoilage; these methods include, chilling, freezing salting, drying, smoking, canning, irradiation, antibacterial, antibiotics microbial food additives and /or their combination of any one with the other (Robergs, Ghiasvand, Parker, 2004). Antibacterial substances are effective against only a few types of bacteria, others are effective against a wide range of organisms, and are known as "board spectrum antibacterial. Lactic acid is primarily found in sour milk products, such as koumiss, leban, yogurt, kefir and some cottage cheeses. The casein in fermented milk is coagulated (curdled) by lactic acid (Robergs, Ghiasvand, Parker, 2004). Although it can be fermented from lactose (milk sugar), most commercially used lactic acid is derived by using bacteria such as *Lactobacillus bulgaricus* to ferment carbohydrates from nondairy sources such as cornstarch, potatoes and molasses. Due to the significant economic losses that spillage causes of food and beverages, it has gained an increasing interest of food microbiologists. Quantitative data about these losses, however, are difficult to obtain. Mainly for reasons of commercial confidentiality, the incidence and the economic costs of spoilage outbreaks are often not reported. Prevention of this spoilage in food products such as fish is addition of chemical preservatives such as alone acetic and alone lactic acids. Because of the consumers increasing awareness towards these preservatives. Growth/no growth *Listeria monocytogenes* and *Escherichia coli*, particularly for seafood productions, It was observed that lactic acid and acetic acid had an inhibitory activity on the growth rate of *L. monocytogenes* and *Escherichia coli.* At 2.0% (w/v), the antimicrobial effect of acetic acid is higher than that of lactic acid. The shelf-life of food will be much longer or the sensorial properties will change as higher amounts of acid are needed (Vermeulen *et al.,* 2007b). Organic acids (acetic acid, citric acid

and lactic acid and/or propionic acid), either singly or in combination used to inhibition *Listeria monocytogenes* and *Escherichia coli* in seafood (Young, and Foegeding, 1993). Acetic and lactic acids are for improving food safety and shelf life of green shell mussels (*Perna canaliculus*). Generally mussels are marinating in 2%acetic and lactic acids for 30 m and placed on vacuum packing and storage at 5ºC. Organic acids (acetic and lactic) are inhibitory to *L. monocytogenes* in mussels (Conner and Bernard, 1990). The antibacterial activity of organic acids is related to the reduction of pH as well as their ability to dissociate, which is determined by the pKa-value of the respective acid, and the pH of the surrounding milieu. The antibacterial activity of organic acids such as lactic acid increases with decreasing pH-value (Russell and Diez-Gonzalez, 1998). Bicarbonate salts are widely available, inexpensive, easy to handle and generally recognized as safe for use in foods (Montville and Goldstein, 1989). They are also commonly used as food additives and can be used in several foodstuffs at 'quantum satis' levels in European and North American regulations (Lindsay, 1996). They therefore potentially present an attractive alternative to more expensive chemical fungicides. Their potential to control fungal growth has been reported by (El-Nabarawy *et al.* 1989), Montville and Goldstein (1989) and Montville and Shih (1991). They have also been found to have an inhibitory effect on the production of trichothecene mycotoxins (Ricke,2003), aflatoxins and ochratoxin A (Montville and Goldstein, 1989). However, no reports on the effect of bicarbonate salts on both growth and fumonisin production exist to date. Moreover the studies to date have investigated bicarbonate impact at one water activity (aw) or moisture content value, neglecting the possible interaction of the effects of aw/moisture content and bicarbonate salts on growth.

Modified Atmospheric Packaging (MAP)

Definition of MAP

Modified atmosphere packaging (MAP) is an increasingly popular food preservation technique. Consumer demands for fresh and convenient foods free of chemical preservation has led to expanding the use of MAP and this technique may also reduce wastage and extends shelf-life of a range of seafood (Church, 1998). MAP techniques have been used for many years to increase the shelf life of many products. This type of packaging has recently become more common due to consumer demands for fresh foods with a prolonged shelf life. MAP is a change in the gaseous atmosphere surrounding the product, still allowing respiration to occur and naturally alter the package environment (Church, 1998). MAP and the addition of antimicrobials have become means of extending the shelf life of ready-to-eat food products (Nilsson *et al*., 2000).

Bacterial inhibition by MAP.

Growth of *L. monocytogenes* in vacuum-packaged products, such as frankfurters has also been documented at temperatures as low as 4°C. A lack of *L. monocytogenes* growth was also seen in vacuum packaged Canadian retail wieners during 28 days of storage at 5°C (Morales *et al*., 2006). Found that fresh fish products stored under vacuum packaging (VP) had an overall increase of shelf life of 7 days after aerobically stored fish. Cann *et al.* (1983) indicated that sensory evaluation limited the MAP shelf life of herring to 8 days, whereas 13 days was obtained with VP. (Randell *et al.*,1997) observed that the sensory quality of both trout and herring fillets deteriorated faster in over-wrap and vacuum packages than MAP.

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