

Fish Spoilage Mechanisms and Preservation Techniques: Review

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Abstract: Problem statement: Spoilage of food products is due to chemical, enzymatic or microbial activities. One-fourth of the world's food supply and 30% of landed fish are lost through microbial activity alone. With the ever growing world population and the need to store and transport the food from one place to another where it is needed, food preservation becomes necessary in order to increase its shelf life and maintain its nutritional value, texture and flavor. The freshness and quality of fish have always gained the attention by Food Regulatory Agencies and Food Processing Industry. Proper handling, pretreatment and preservation techniques can improve the quality fish and fish products and increase their shelf life. **Methodology:** Historically salting, drying, smoking, fermentation and canning were the methods to prevent fish spoilage and extend its shelf life. In response to consumer demand for texture, appearance and taste, new methods were developed including: Cooling, freezing and chemical preservation. A comprehensive review of the literature on the subject of fish spoilage and modern preservation techniques was carried out. **Conclusion:** Fish spoilage results from three basic mechanisms: Enzymatic autolysis, oxidation, microbial growth. Low temperature storage and chemical techniques for controlling water activity, enzymatic, oxidative and microbial spoilage are the most common in the industry today. A process involving the addition of an EDTA (1 mM)-TBHQ (0.02%) combination and ascorbic acid and storage at refrigerated temperatures (5°C) in darkness can be the most positive for controlling the spoilage of fish and fish product. The suggested process would address antimicrobial activity as well as destructive oxidation of the desired lipids and fats. However, more efforts are required to understand the role of proximate composition of fish, post harvest history, environmental conditions, initial microbial load, type and nature of bacteria and their interaction in order to optimize the shelf-life of fish.

Key words: Fish, spoilage, shelf-life, low temperature storage, chemical preservation

INTRODUCTION

With the ever growing world population and the need to store and transport the food from one place to another where it is needed, food preservation becomes necessary in order to increase its shelf life and maintain its nutritional value, texture and flavor. Therefore, good food preservation techniques must prevent microbial spoilage of food without affecting its quality and nutritional.

Kader (2005) and Harvey (1978) stated that about one third of all fruits and vegetables produced worldwide are lost due to spoilage. NAS (1978) estimated a 10% loss of cereals in developing countries. Kantor *et al.* (1997) conducted a detailed study on food losses in during total retail, foodservice and consuming in USA and reported total losses of 23, 24, 15 and 30% for fruits, vegetables, meat, poultry and fish products

and dairy products, respectively as shown in Table 1. Swanton *et al.* (1993) estimated the average annual cost of crop loss in Canada to be over \$984 million dollars. A value of \$4 billion of perishables food losses was estimated and more than \$11 billion of total food losses were also estimated in developing countries.

Spoilage of food products can be due to chemical, enzymatic or microbial activities. Chemical deterioration and microbial spoilage are responsible for loss of 25% of gross primary agricultural and fishery products every year (Baird-Parker, 2000). One-fourth of the world's food supply (Huis in't Veld, 1996) and 30% of landed fish (Amos, 2007) are lost through microbial activity alone. Around 4-5 million tons of trawled and shrimp fish are lost every year due to enzymatic and microbial spoilage because of improper onsite storage (Unklesbay, 1992).

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Table 1: Food losses at retail, foodservice and consumer levels in USA in 1995 (Kantor *et al.*, 1997)

Commodity	Edible food supply	Losses from edible food supply			
		Retail food loss		Food service and consumer food loss	
	Million pounds	Million pounds	(%)	Million pounds	(%)
Grain products	45,606	912	2	13,682	30
Fruit					
Fresh	22,389	448	2	6,717	30
Processed	25,949	259	1	3,892	15
Total	48,338	707	2	10,609	23
Vegetables					
Fresh	36,830	737	2	11,049	30
Processed	26,247	262	1	3,898	15
Total	63,077	999	2	14,947	24
Meat, poultry and fish					
Red meat	30,350	303	1	4,552	15
Poultry	17,108	171	1	2,566	15
Fish and seafood	4,008	40	1	601	15
Total	51,466	515	1	7,720	15
Dairy products					
Fluid milk	54,474	1,089	2	16,342	30
Other	21,802	436	2	6,541	30
Total	76,276	1,525	2	22,883	30

Fresh fish spoilage can be very rapid after it is caught. The spoilage process (Rigor mortis) will start within 12 h of their catch in the high ambient temperatures of the tropics (Berkel *et al.*, 2004). Rigor mortis is the process through which fish loses its flexibility due to stiffening of fish muscles after few hour of its death (Adebowale *et al.*, 2008). Most fish species degrade as a result of digestive enzymes and lipases, microbial spoilage from surface bacteria and oxidation (AMEC, 2003). During fish spoilage, there is a breakdown of various components and the formation of new compounds. These new compounds are responsible for the changes in odour, flavor and texture of the fish meat.

This represents a major concern of the freshness of saleable products and the breakdown of proteins and lipids. Higher energy demanding freeze-storage preservation can be altered by synthetic or natural preservatives for control of lipid oxidation and microbial growth in fish during storage (Mahmoud *et al.*, 2006). Combination of these preservatives and refrigeration diminishes the process of spoilage (Bagamboula *et al.*, 2004).

Compositional changes during fish spoilage result in lipid oxidation and protein degradation as well as the loss of other valuable molecules. In order to develop optimum preservation techniques for these value added products in active forms, understanding of the mechanism responsible for their degradation is essential. This review will focus on basic mechanisms of fish spoilage, preservation of fish with low temperature storage and comprehensive analysis of chemical preservation methods.

FISH SPOILAGE MECHANIZMS

Fish spoilage results from three basic mechanisms: Enzymatic autolysis, oxidation, microbial growth.

Autolytic enzymatic spoilage: Shortly after capture, chemical and biological changes take place in dead fish due to enzymatic breakdown of major fish molecules (FAO, 2005). Hansen *et al.* (1996) stated that autolytic enzymes reduced textural quality during early stages of deterioration but did not produce the characteristic spoilage off-odors and off-flavors. This indicates that autolytic degradation can limit shelf-life and product quality even with relatively low levels of spoilage organisms. The autolytic changes that occur in chilled/frozen fish are summarized in Table 2 (FAO, 2005). Most of the impact is on textural quality along with the production of hypoxanthine and formaldehyde. The digestive enzymes cause extensive autolysis which results in meat softening, rupture of the belly wall and drain out of the blood water which contains both protein and oil (FAO, 1986).

A number of proteolytic enzymes are found in muscle and viscera of the fish after catch. These enzymes contribute to post mortem degradation in fish muscle and fish products during storage and processing. There is a sensorial or product associated alteration that can be contributed by proteolytic enzymes (Engvang and Nielsen, 2001). During improper storage of whole fish, proteolysis is responsible for degradation of proteins and is followed by a process of solubilization (Lin and Park, 1996). On the other hand, peptides and free amino acids can be produced as a result of autolysis of fish muscle proteins, which lead towards the spoilage of fish meat as an outcome of microbial growth and production of biogenic amines (Fraser and Sumar, 1998). Belly bursting is caused by leakage of proteolytic enzymes from pyloric caeca and intestine to the ventral muscle. The proteases have optimal pH in the alkaline to neutral range. Martinez and Gildberg (1988) reported that the rate of degradation by proteolytic enzymes was reduced when the fish was kept at 0°C and a pH of 5.

Table 2: Summary of changes in chilled or frozen fish (FAO, 2005)

Enzyme(s)	Substrate	Effect	Prevention
Glycolytic enzymes	Glycogen	Lactic acid production resulting in pH drop.	Avoid pre-rigor stress
Autolytic enzymes involved in nucleotide breakdown	ATO, ADP, AMP, IMP	Gradual production of Hypoxanthine	Avoid pre-rigor stress and improved handling.
Cathepsins	Proteins, peptides	Softening of tissue	Avoid rough handling during storage
Chymotrypsin, trypsin, carboxy-peptidases	Proteins, peptides	Belly-bursting	Problem increased with freezing/thawing or long-term chill storage
Calpain	Myofibrillar proteins	Softening	Removal of calcium
Collagenases	Connective tissue	Softening and gaping of tissue	Time and temperature of chilled storage
Trimethylamine Oxide (TMAO) demethylase	TMAO	Formaldehyde	Storage temperature less than -30°C, physical abuse, freeze/thawing

Aoki and Ueno (1998) reported that the major cause of post-mortem degradation of mackerel muscle tissue was due to the activity of Cathepsin L. The enzyme was liberated from lysosomes during ageing for 7 days at 0°C. The isolated enzyme was hydrolyzed to myosin, troponin T, troponin I and tropomyosin. This hydrolytic action of mackerel cathepsin L was similar to that of cathepsin L from chum salmon white muscle as reported by Yamashita and Konagaya (1990). Yongsawatdigul *et al.* (2000) reported proteolytic degradation of tropical tilapia surimi when the temperature was increased beyond 40°C with a maximum activity at 65°C. Due to inhibition by soybean trypsin inhibitor and leupeptin it was implied that the proteinase involved was serine proteinase.

Oxidative spoilage: Lipid oxidation is a major cause of deterioration and spoilage for the pelagic fish species such as mackerel and herring with high oil/fat content stored fat in their flesh (Fraser and Sumar, 1998). Lipid oxidation involves a three stage free radical mechanism: initiation, propagation and termination (Frankel, 1985; Khayat and Schwall, 1983). Initiation involves the formation of lipid free radicals through catalysts such as heat, metal ions and irradiation. These free radicals which react with oxygen to form peroxy radicals. During propagation, the peroxy radicals reacting with other lipid molecules to form hydroperoxides and a new free radical (Fraser and Sumar, 1998; Hultin, 1994). Termination occurs when a build up of these free radicals interact to form non-radical products. Oxidation typically involves the reaction of oxygen with the double bonds of fatty acids. Therefore, fish lipids which consist of polyunsaturated fatty acids are highly susceptible to oxidation. Molecular oxygen needs to be activated in order to allow oxidation to occur. Transition metals are primary activators of molecular oxygen (Hultin, 1994).

In fish, lipid oxidation can occur enzymatically or non-enzymatically. The enzymatic hydrolysis of fats by lipases is termed lipolysis (fat deterioration). During

this process, lipases split the glycerides forming free fatty acids which are responsible for: (a) common off-flavour, frequently referred to as rancidity and (b) reducing the oil quality (Huis in't Veld, 1996; FAO, 1986). The lipolytic enzymes could either be endogenous of the food product (such as milk) or derived from psychrotrophic microorganisms (Huis in't Veld, 1996). The enzymes involved are the lipases present in the skin, blood and tissue. The main enzymes in fish lipid hydrolysis are triacyl lipase, phospholipase A2 and phospholipase B (Audley *et al.*, 1978; Yorkowski and Brockerhoff, 1965).

Non-enzymatic oxidation is caused by hematin compounds (hemoglobin, myoglobin and cytochrome) catalysis producing hydroperoxides (Fraser and Sumar, 1998). The fatty acids formed during hydrolysis of fish lipids interact with sarcoplasmic and myofibrillar proteins causing denaturation (Anderson and Ravesi, 1969; King *et al.*, 1962). Undeland *et al.* (2005) reported that lipid oxidation can occur in fish muscle due to the highly pro-oxidative Hemoglobin (Hb), specifically if it is deoxygenated and/or oxidized. They found that the addition of acids, which lower the pH, can accelerate lipid oxidation through deoxygenated Hb.

Microbial spoilage: Composition of the microflora on newly caught fish depends on the microbial contents of the water in which the fish live. Fish microflora includes bacterial species such as *Pseudomonas*, *Alcaligenes*, *Vibrio*, *Serratia* and *Micrococcus* (Gram and Huss, 2000) Microbial growth and metabolism is a major cause of fish spoilage which produce amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable off-flavors (Dalgaard *et al.*, 2006; Emborg *et al.*, 2005; Gram and Dalgaard, 2002). For unpreserved fish, spoilage is a result of Gram-negative, fermentative bacteria (such as *Vibrionaceae*), whereas psychrotolerant Gram-negative bacteria (such as *Pseudomonas* spp. and *Shewanella* spp.) tend to spoil

chilled fish (Gram and Huss, 2000). It is, therefore, important to distinguish non spoilage microflora from spoilage bacteria as many of the bacteria present do not actually contribute to spoilage (Huss, 1995). The compounds formed during spoilage through microbial metabolism are listed in Table 3.

Trimethylamine (TMA) levels are used universally to determine microbial deterioration leading to fish spoilage. Fish use Trimethylamine Oxide (TMAO) as an osmoregulant to avoid dehydration in marine environments and tissue waterlogging in fresh water. Bacteria such as *Shewanella putrefaciens*, *Aeromonas* spp., *psychrotolerant Enterobacteriaceae*, *P. phosphoreum* and *Vibrio* spp. can obtain energy by reducing TMAO to TMA creating the ammonia-like off-flavors (Gram and Dalgaard, 2002). Table 4 lists spoilage bacteria in descending order of spoilage activity. *Pseudomonas putrefaciens*, *fluorescent pseudomonads* and other spoilage bacteria increase rapidly during the initial stages of spoilage, producing many proteolytic and hydrolytic enzymes (Shewan, 1961).

Table 3: Bacterial spoilage compounds (Church, 1998)

Specific spoilage bacteria	Spoilage compounds
<i>Shewanella putrefaciens</i>	TMA, H ₂ S, CH ₃ SH, (CH ₃) ₂ S, HX
<i>Photobacterium phosphoreum</i>	TMA, HX
<i>Pseudomonas</i> spp.	Ketones, aldehydes, esters, non -H ₂ S sulphides
<i>Vibrionaceae</i>	TMA, H ₂ S
Aerobic spoilers	NH ₃ , acetic, butyric and propionic acid

TMA: Trimethylamine; H₂S: Hydrogen sulphide; CH₃SH: Methylmercaptan; (CH₃)₂S: Dimethylsulphide; HX = Hypoxanthine
NH₃: Ammonia

Table 4: Spoilage activity of bacteria (Hui, 1992)

Spoilage activity	Microorganism
High	<i>Pseudomonas (Alteromonas) putrefaciens</i> <i>Pseudomonas (altreomonas) fluorescens</i> Fluorescent pseudomonads
Moderate	<i>Moraxella</i> , <i>Acinetobacter</i> and <i>Alcaligenes</i>
Low (Specific conditions)	<i>Aerobacter</i> , <i>Lactobacillus</i> , <i>Flavobacterium</i> , <i>Micrococcus</i> , <i>Bacillus</i> and <i>Staphylococcus</i>

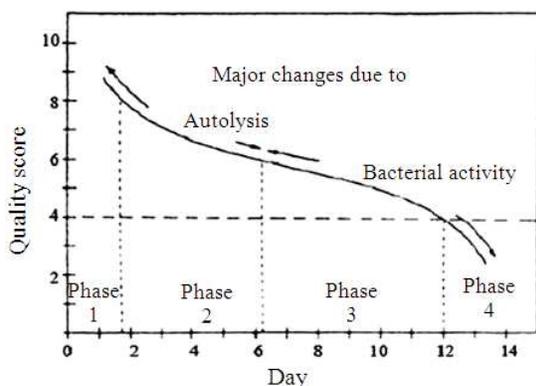


Fig. 1: The four phases of fish spoilage (Shawyer and Pizzali, 2003)

Liston (1980) stated that proteins are degraded by the proteases into peptides and amino acids, whereas lipids are degraded by lipases into fatty acids, glycerols and other compounds. Figure 1 illustrates spoilage of fish in four phases which starts form autolytic deterioration caused by enzymes and is responsible for initial first two phases of spoilage. The other two phases of spoilage result from the microbes, mainly bacteria which finally spoil the fish to such an extent that it cannot be edible (Abbas *et al.*, 2009).

Olafsdottir *et al.* (2006) reported on the proliferation of specific spoilage organisms in haddock fillets stored at 0, 7 and 15°C and found *Photobacterium phosphoreum* to be predominant among spoilage bacteria. *Pseudomonas* spp. appeared responsible for sweet, fruity spoilage odors while *Shewanella putrefaciens* was responsible for the H₂S production. Nuin *et al.* (2008) reported on the growth of spoilage bacteria on farmed turbot at temperatures of 0 and 15°C. As shown in Fig. 2, *Pseudomonas* spp. showed the fastest growth at both temperatures and was considered responsible for turbot spoilage.

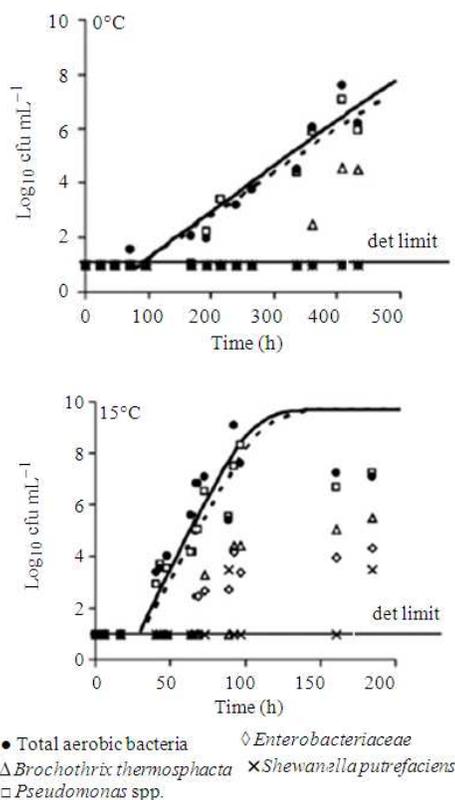


Fig. 2: Concentration of spoilage bacteria in farmed turbot: Line-Total aerobic bacteria, Dashed-*Pseudomonas* spp. (Nuin *et al.*, 2008)

FISH PRESERVATION TECHNIQUES

Different types of preservation methods such as drying, smoking, freezing, chilling, brining, fermentation and canning are reported to extend the self-life of seafoods and meat products. However, low temperature storage and chemical techniques for controlling water activity, enzymatic, oxidative and microbial spoilage are the most common in the industry today (Akinola *et al.*, 2006; Berkel *et al.*, 2004).

Low temperature storage: Since mid of 19th century, the low temperature storage method have been used for the preservation of wide varieties of seafood's which retard the growth of microorganisms. This method of preservation does not kill the microorganisms but reduces microbial metabolism which is responsible for spoilage (Ashie *et al.*, 1996). Johnston *et al.* (1994) stated that freezing and cold storage are efficient methods of fish preservation but they do not improve product quality. It is necessary to preserve the fish at 0°C after catch as its spoilage is very rapid (FAO, 1973). Berkel *et al.* (2004) reported two possibilities for storing fresh fish at low temperatures: (a) cooling at -1° to +4°C, which inhibits the growth of microorganisms and (b) freezing at -18 to -30°C, which completely stops bacteria from growing. However, both enzymatic and non enzymatic changes continue but at a much slower rate.

The use of ice or other methods of chilling is recommended to keep the fish all times in a cool condition before freezing (Johnston *et al.*, 1994). The functions of ice include: (a) maintaining uniform low temperature, (b) reducing autolysis and bacterial degradation and (c) providing a gentle washing/cleaning effect during melting (Rand and Pivarnik, 1992). In order to get the temperature below the freezing point of pure water, ice is made from saltwater to achieve a chilled temperature close to -2.5°C. However, a possible reduction of flavor due to loss of free amino acids may be a problem (Borgstrom, 1968). Freezing operation for preservation cannot ensure the prevention of amino acid losses. However, it surely impedes the physical and biochemical reactions which are responsible for spoilage of food (George, 1993). Survival of spoilage microorganisms during storage depends on the types of microorganisms and fish species, history of the fish, methods of catch and the handling and storage processes aboard the fishing vessel (Ashie *et al.*, 1996).

Fish contains about 60-80% by weight water depending on the species. The process of freezing converts most of water into ice (Johnston *et al.*, 1994).

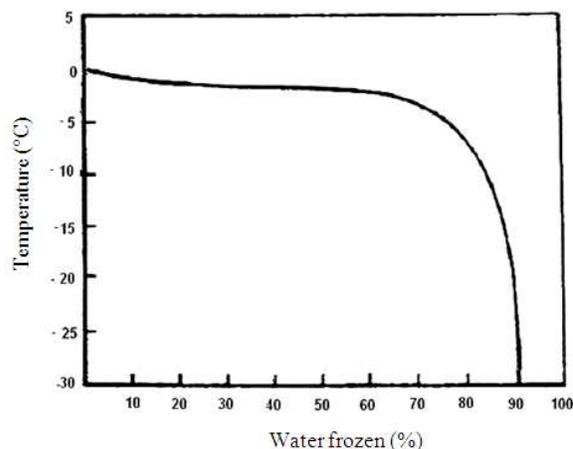


Fig. 3: Percent of frozen water during freezing of fish at different temperatures (Johnston *et al.*, 1994)

Even though freezing point of fish is -1 to -2°C, at a temperature of -25°C only 90-95% of water freezes. This does not include bound water which is chemically bound to specific sites such as carbonyl and amino group of proteins and hydrogen bonding (Garthwaite, 1997). Johnston *et al.* (1994) reported on the variation of the proportion of water (which is converted to ice) in the muscle tissue of fish against temperature. As shown in Fig. 3, 70% of the water gets frozen at -5°C. However, at temperatures as low as -30°C, a proportion of the water in the fish muscle still remains in the unfrozen state. The final quality depends on the quality of the fish at the time of freezing as well as other factors including: freezing/cold storage temperature, freezing rate and distribution (Johnston *et al.*, 1994).

The most crucial factor which affects the quality of frozen fish is the freezing rate (slow and fast). Fast freezing produces better quality frozen fish than slow freezing as slow freezing results in the formation of large ice crystals, in comparison to fast freezing, which damage the walls of the cells and cause denaturation of protein. On other hand, denaturation also depends on the concentration of enzymes and other compounds present (Rahman, 1999; Garthwaite, 1997; Johnston *et al.*, 1994). The change of proteins results in dull and opaque texture and the tissue becomes soft and spongy which severely affects the quality of fish product (Graham, 1982).

The enzymatic reactions can still continue in frozen fish at a temperature of -30°C (Cassens, 1994; Graham, 1982) which involve a number of other metabolites: Glycolysis, nucleotide degradation and proteolysis (Jones, 1963). These endogenous enzymatic activities cause intrinsic chemical and physical changes.

Although enzymatic activities are slow, they can still support microbial growth and metabolism (Simpson, 1997). The cold storage life of some fish products at -30°C is summarized by Graham (1982) in Table 5.

Microbial spoilage impedes at a recommended storage temperature of -9 to -12°C. However, enzyme present in the fish will still play an important part in spoilage. About 10-60% of the viable microbial population die during freezing yet the remaining population gradually increase during frozen storage (Rahman, 1999). Morlier *et al.* (1989) reported that *Anasakis simplex*, a nematode found in certain fish, can be killed by freezing to -20°C and holding at least 24 h. Raj and Liston (1961) reported that only a 1 log₁₀ reduction in numbers of *Salmonella typhimurium* when fish was frozen at -22°C and stored at -17.9°C for over 1 year. Miladi *et al.* (2008) found that freeze storage of *L. monocytogenes* in salmon does not decrease the potential survival of food-borne pathogens over a period of ten months at freezing temperature of -20°C. During cold storage, Thimethylamine Oxide (TMAO) decomposition in fish and seafood results in to formation of trimethylamine and dimethylamine which are responsible for off flavor (Hui, 2006) and formaldehyde reacts with proteins and is responsible for decreasing their solubility in salt and buffer solutions (Rahman, 1999). Arannilewa *et al.* (2005) investigated the effect of duration of freeze storage on the chemical, microbiological and sensory profile of tilapia fish (*Sarotherodon galiaenus*). They reported decreases in the values of protein and fat by 27.9 and 25.92%, respectively. The total coliform count was increased from 3.0×10³-7.5×10⁶ during storage.

The freezing of fish is achieved using different freezing methods. The basic principles include: Blowing cooled air over fish, placing fish in contact with cooled metal plate and by immersing fish in a low temperature liquid. The advantages and disadvantages of different methods are summarized in Table 6.

Controlling water activity: Although freezing is the best way to inhibit microbial growth (except psychrophiles and spores) and slow chemical changes

thereby preserving fish for a long time, it is an energy intensive operation and can only be used as a temporary method of preservation. Most of the psychrophiles and spores survive during freezing and grow during thawing. Also, freezing does not prevent oxidative spoilage as enzymatic spoilage continues at slower rate (Neumeyer *et al.*, 1997). Fish spoilage can be prevented by controlling water activity. For the growth of every microorganism there are minimum, optimum and maximum water activity same like pH and temperature. Therefore, lowering water activity (a_w) can minimize putrefaction and improve preservation of fish (Abbas *et al.*, 2009). The term water activity (a_w) refers to the water which is not bound to food molecules and can support the growth of bacteria, yeasts and moulds (fungi). The water activity (a_w) represents the ratio of the water vapor pressure of the food to the water vapor pressure of pure water under the same conditions and it is expressed as a fraction (CSIRO, 2005). The control of water activity in fish is accomplished by drying, adding chemicals, or a combination of both methods. Sugars and sodium chloride have been used to bind up the free water molecules and create an osmotic imbalance resulting in cell growth inhibition (Ray, 2004).

Table 5: The cold storage life of some fish products at -30°C (Graham, 1982)

Item	Storage life (months)
White fish (guttled)	8.0
White fish (smoked)	7.0
Herring (guttled)	6.0
Herring (unguttled)	6.0
Mackerel (guttled)	6.0
Mackerel (unguttled)	6.0
Kippers	4.5
Raw whole nephrops	8.0
Shelled nephrops	8.0
Cooked shucked mussels	8.0
Raw shrimp	6.0
Raw whole oysters	6.0
Scallop meat	6.0
Cooked shrimp	6.0
Cooked whole crab	6.0
Cooked whole lobster	6.0
Extracted crab meat	4.0

Table 6: Comparison of common freezing methods

Freezing methods	Description	Advantages	Disadvantages
Air blast freezer	Materials are frozen by ventilated cooled air while passing slowly on moving belts in a tunnel	Accept wide range of shapes and sizes of materials to be frozen	Easy to be used inefficiently and incorrectly and running cost is high
Plate freezer	The article to be frozen is placed the middle of two refrigerated plates	Consume less power and occupies less space than air blast freezer	Poor contact between materials and plates and is effective only on flat packages
Immersion freezing	Direct immersion of the material into cold liquid environment	Very efficient	Difficulties in finding suitable liquid which has low freezing point and does not contaminate food
Liquid nitrogen freezing	Using liquid nitrogen at 196°C to - spray on the fish to be frozen	Short freezing time; compact equipment and low product weight loss	High cost of liquid nitrogen and the potential danger involved when using liquid nitrogen

Table 7: Minimal water activity for sodium chloride and glycerol (Chirife, 1994)

Bacteria	Sodium chloride (a_w)	Glycerol (a_w)
<i>Clostridium botulinum</i> E	0.965	0.940
<i>Clostridium botulinum</i> A	0.955	0.935
<i>Clostridium perfringens</i>	0.947	0.930
<i>Vibrio parahaemolyticus</i>	0.933	0.910
<i>Listeria monocytogenes</i>	0.920	0.900

Table 8: Minimal water activity for *S. aureus* growth in various solutes (Ballesteros *et al.*, 1993)

Solute	Minimal water activity (a_w)
Ethanol	0.975
Propylene glycol	0.960
Glycerol	0.890
Sucrose	0.870
Sodium chloride	0.860

Sperber (1983) reported that the minimum value of a_w required for bacterial growth ranged from 0.99 for *Moraxella/Acinetobacter* type organism to 0.86 for *Staphylococcus aureus*. Since that time, hurdle technology emerged based on an application of combined preservative factors (called hurdles) to prevent microbial growth and enhance shelf-life (Leistner and Gorris, 1995). The most important hurdles used in food preservation are: temperature (high or low), water activity (a_w), acidity (pH), redox potential (Eh), preservatives (nitrite, sorbate, sulfite) and competitive microorganisms such as lactic acid bacteria (Leistner, 2000).

Chawla *et al.* (2006) reported a reduction in water activity of fresh lamb intestine from 0.95-0.80 with the addition of 10% (w/w) of sodium chloride as part of a hurdle technology. The major setback for the use of melerates the development of lipid oxidation and thus sodium chloride is that its prooxidant activity which accelerates the development of lipid oxidation and thus the deterioration of value added products (Decker and Xu, 1998).

Chirife (1994) reported that the inhibitory effects of sucrose and sodium chloride on *Staphylococcus aureus* were primarily related to a lower water activity. He suggested that other solutes such as ethanol, propylene glycol, butylenes glycol and various polyethylene glycols may have antibacterial effects related mainly to interaction with membrane enzymes responsible for peptidoglycan synthesis. Table 7 compares the minimal water activity for growth of various pathogenic bacteria using sodium chloride or glycerol. In all cases sodium chloride is more inhibitory than glycerol. Ballesteros *et al.* (1993) reported that the minimal water activity for *Staphylococcus aureus* growth of various solutes ranged from 0.86-0.975 as shown in Table 8. They observed that solutes such as alcohol, diols and polyethylene glycols are able to

inhibit growth at much higher water activity than sodium chloride and sucrose.

Controlling autolytic enzymatic spoilage: As the fish degradation process begins with autolytic activity, it is important to slow the action of the digestive enzymes to improve preservation. This can be accomplished by removing the enzymes or by developing techniques that inhibit their activities. Gutting of the fish immediately after capture can avoid the invasion of digestive tract proteases through the abdominal cavity to the tissue and prevent or slow degradation (Pedrosa-Menabrito and Regenstein, 1988).

Gutting: In order to remove the strong proteases from the digestive track, gutting of the fish immediately after catch is essential. This procedure needs to be performed properly in order to avoid contact of digestive enzymes into tissue (Pedrosa-Menabrito and Regenstein, 1988). It should be noted that many factors such as the age of the fish, the species, the amount of lipid, the catching ground and method of catch will determine weather gutting of fish could be advantageous or not. Fatty fish species such as herring, sardine and mackerel are susceptible to heavy autolytic degradation when caught just after feeding. However, the gutting of lean species such as cod, is compulsory (Huss, 1988). The increased stomach and gut content have accompanying increased levels of digestive enzymes (Pedrosa-Menabrito and Regenstein, 1988). The digestive enzymes leak out during post-mortem when the belly walls burst (Gildberg and Raa, 1980). Belly bursting can be limited if the fish are kept cool and processed (gutted) quickly after catching (Hobbs, 1982). However, there may be chances of bacterial cross-contamination of fish during the gutting procedure (Erkan and Ozden, 2006).

Addition of sodium chloride: Sodium chloride has shown the ability to inactivate autolytic enzymes in marine species. Reddi *et al.* (1972) reported the inactivation of cathepsin extracted from winter flounder using 5% NaCl when incubated for 25 h at room temperature. They concluded that NaCl is an inactivator of catheptic activity, which may, also, control autolytic spoilage. The effect of NaCl on cathepsin is shown in Fig. 4.

Siringan *et al.* (2006) reported extensively on the autolytic activity of endogenous proteinases in Indian anchovy. They found that 25% (w/w) NaCl reduced autolytic activity by 48% as shown in Fig. 5. Sen (2005) reported that 10% solution of sodium chloride inactivated the autolytic enzyme when deveined shrimp was blanched for 5 minutes at 80°C.

Klomklao *et al.* (2004) studied proteolytic activities of splenic extract from three tuna species: skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacores*) and tonggol tuna (*Thunnus tonggol*). They reported that autolytic activities continuously decreased as NaCl concentration increased and no activity remained in the presence of 30% (w/v) NaCl at 55°C and pH 9.0.

Yongsawatdigul *et al.* (2000) reported that proteolysis of tilapia surimi decreased by about 76% compared to the control (0% NaCl) when surimi was incubated in 1M NaCl at 65°C for 1.5 h as shown in Fig. 6. However, Reddi *et al.* (1972) reported that increasing salt concentrations reduced catheptic activity, but enzymes were still active at salt levels relevant to smoked salmon.

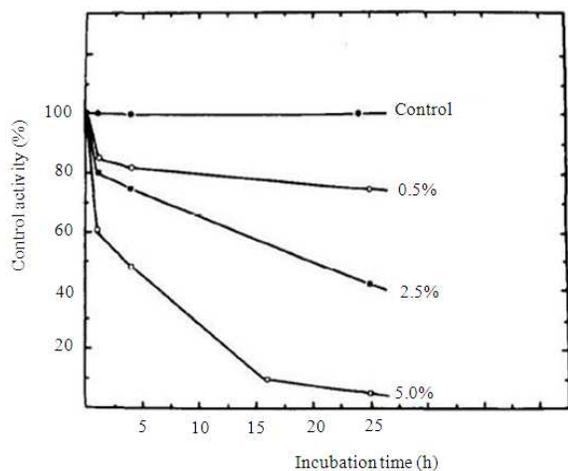


Fig. 4: The effect of NaCl on cathepsin (Reddi *et al.*, 1972)

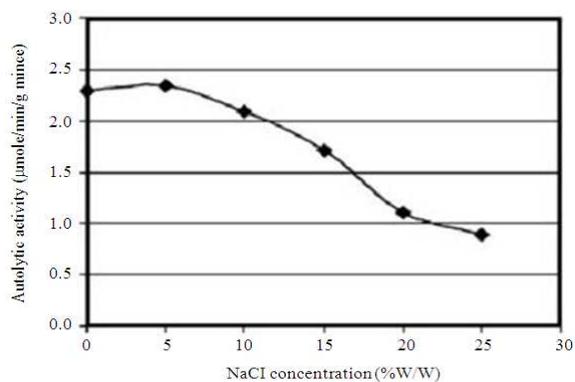


Fig. 5: Effect of NaCl on autolytic activity in Indian anchovy (Siringan *et al.*, 2006)

Addition of acids: Martinez and Gildberg (1988) reported on the rate of Anchovy tissue degradation during storage with reduced pH. The fish were stored in sea water at 1°C with the addition of lactic/acetic acid or propionic acid to lower the pH to 5. After a storage period of 35 h, they found that lactic acid was effective in reducing the percentage of belly bursting by half from 19-10% as illustrated in Table 9.

Hidalgo *et al.* (1999) conducted a comparative study of the proteolytic activities in six species of fish with different nutritional habits: Rainbow trout (*Oncorhynchus mykiss*), gilthead seabream (*Sparus aurata*) European eel (*Anguilla anguilla*), common carp (*Cyprinus carpio*), goldfish (*Carassius auratus*) and tench (*Tinca tinca*). When proteolytic activity was determined at a wide range of pH using 0.1 M KCl-HCl (pH 1.5), 0.2 M glycine-HCl (pH 3.0), 0.1 M citrate (pH 4.0), 0.2 M phosphate (pH 7.0), 0.1 M Tris-HCl (pH 9.0) and 0.1 M glycine-NaOH (pH 10.0), at 25°C, the highest values in the digestive tract of all species were found at alkaline and neutral pHs as shown in Fig. 7. Only very low activity was observed at acidic pHs. An exceptional result was found in the case of eel where proteolytic activity was detected only at acidic pHs. However, Cabrer *et al.* (2002) reported that Cathepsin activity was increased in lower pH of marinated fish in acetic acid.

Controlling oxidative spoilage: To ensure maximum lipid yield, lipid oxidation and inhibition needs to be studied and understood. In order to inhibit lipid oxidation, the free radical mechanism catalysts (molecular oxygen and transition metals) need to be removed.

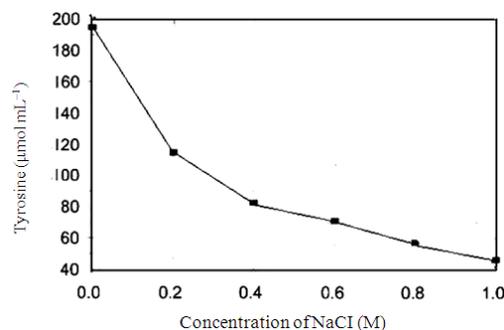


Fig. 6: Effect of NaCl concentration on autolytic activity of tilapia surimi at 65°C (Yongsawatdigul *et al.*, 2000)

Table 9: Influence of acid addition on rate of belly bursting after 35 hrs of storage (Martinez and Gildberg, 1988)

Sample	Initial pH	Final pH	Belly bursting (%)
Control*	8	6.3	19
Acetic acid	5	5.9	11
Lactic acid	5	5.9	10
Propionic acid	5	5.9	13

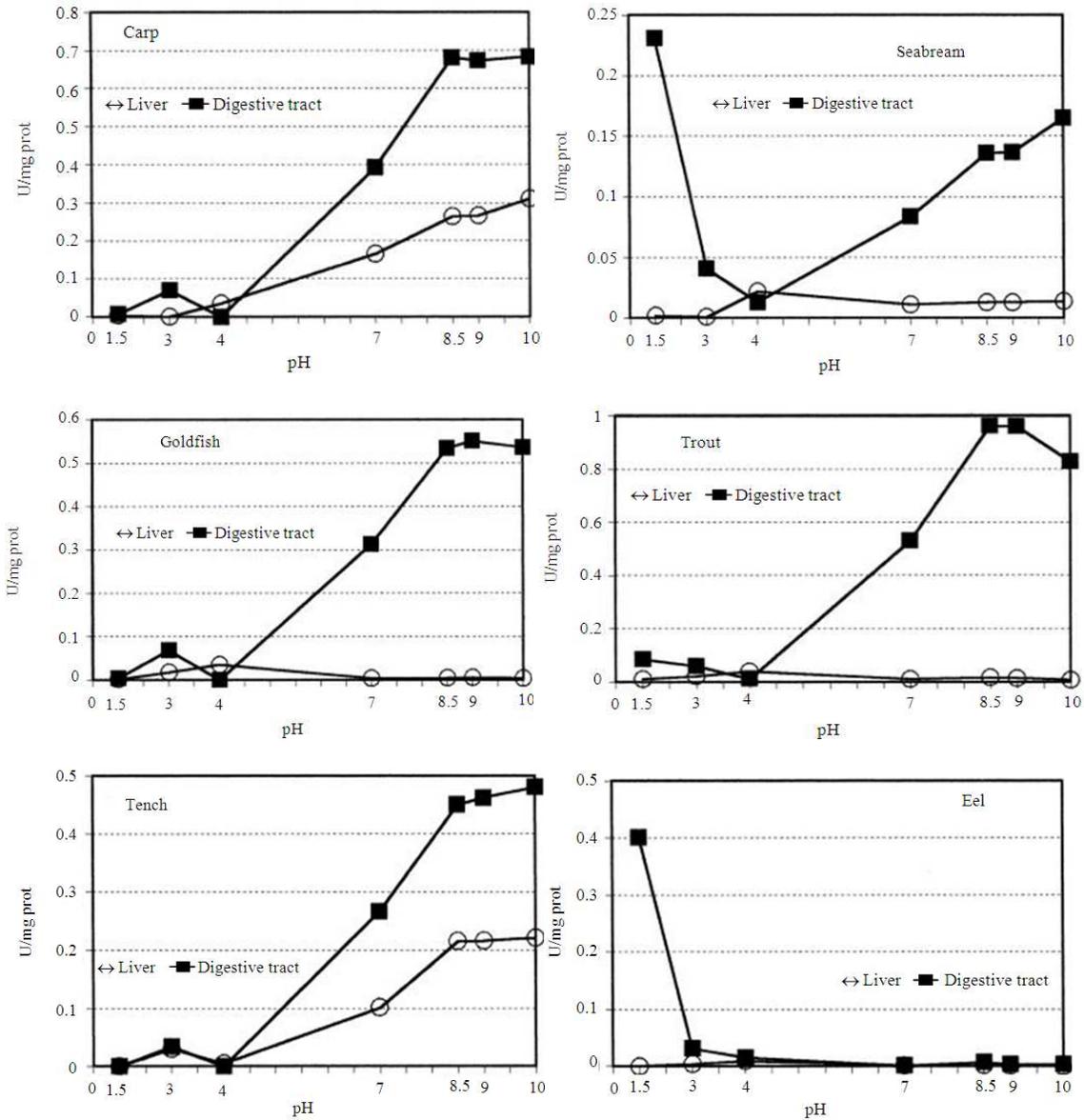


Fig. 7: pH dependence of the proteolytic activity in liver and digestive tract of the different species of fish (Hidalgo *et al.*, 1999)

By removing these catalysts through antioxidants and chelating agents, lipid oxidation can be limited and the lipid yield improved. An index often used to determine lipid oxidation is the measurement of Thiobarbituric Acid Reactive Substances (TBARS). Among lipid oxidation inhibitory additives are: Phenolic antioxidants and ethylenediaminetetraacetic acid.

Phenolic antioxidants: Derivatives of phenol such as Butylated Hydroxyanisole (BHA), Butylated

Hydroxytoluene (BHT) and Tertiary Butylhydroquinone (TBHQ) are referred to as phenolic antioxidants (Davidson, 1993). BHA, BHT and TBHQ have antimicrobial properties against bacteria (predominately gram-negative), fungi, viruses and protozoa (Branen *et al.*, 1980). According to Ray (2004), the antimicrobial activity is due to the adverse effects on cell membranes and enzymes. The antioxidant characteristics of the phenol-derivatives may be of greater benefit in terms of preservation.

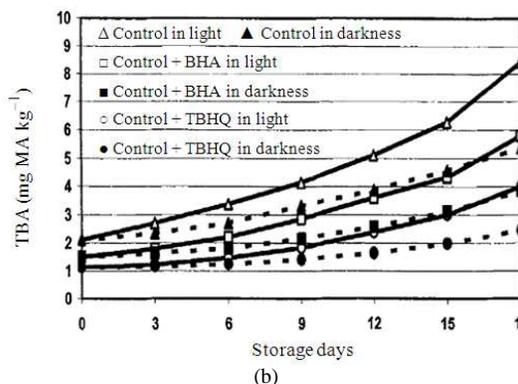
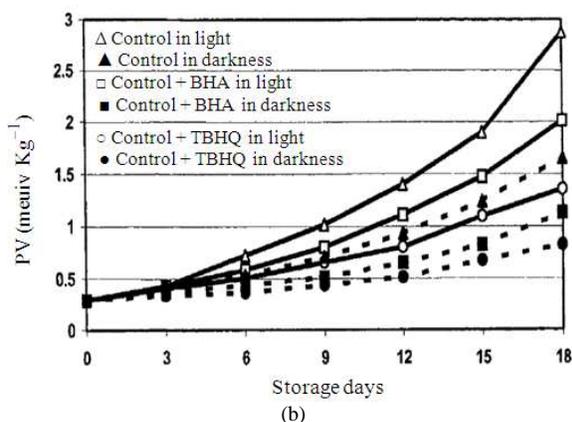
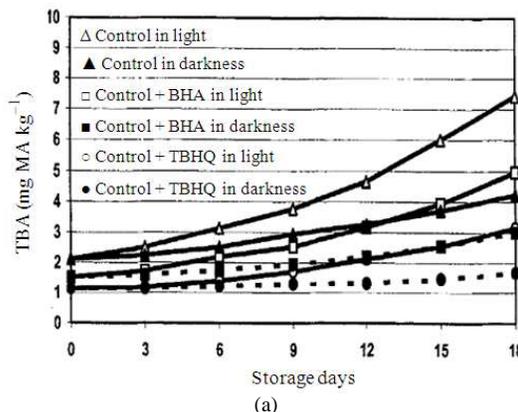
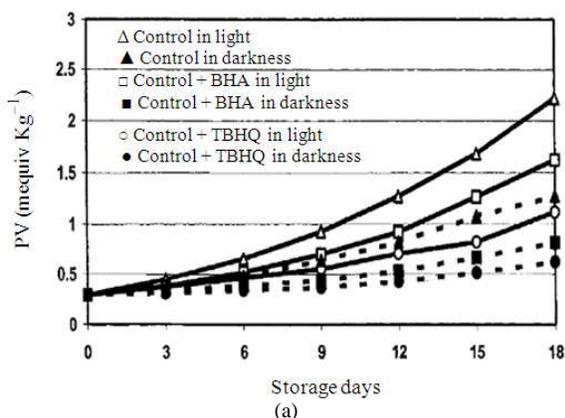


Fig. 8: Peroxide values for crude Mackerel oil during oxidation testing at (a) 5°C and (b) 40°C (Sang and Jin, 2004)

Fig. 9: TBA values for crude Mackerel oil during oxidation testing at (a) 5°C and (b) 40°C (Sang and Jin, 2004)

The antioxidant effectiveness in inhibiting oxidation may be in part due to the number of phenolic hydroxyl groups available for free radical scavenging (Rice-Evans *et al.*, 1996).

The Canadian Food and Drug Act limit the amount of phenolic antioxidants either singly or combined at 0.02% (DJC, 2009). Kaitaranta (1992) found that TBHQ (at 0.01%) to be twice as effective in its antioxidative properties compare to BHA or BHT (at 0.02%). TBHQ had an antioxidant efficiency value of 4.0 where BHA and BHT had values of 1.8 and 2.2, respectively. They, also, reported that during preliminary studies TBHQ had showed practically no measurable oxidation in the accelerated tests at levels higher than 0.02%. Kelleher *et al.* (1992) reported similar results.

Sang and Jin (2004) reported on a combination of photo-oxidative stress and antioxidant activity of BHA and TBHQ in crude and refined Mackerel liver oil.

Changes in Peroxide Value (PV) and TBARS (thiobarbituric acid reactive substances) were monitored over 18 days at 5 and 40°C. The peroxide value is used as an indicator of the extent of oxidative rancidity. It is affected by the age of raw material as well as oxidation of lipids during processing and storage (AMT, 1997). The results showed that exposure to visible light played the most critical role in oxidation followed by BHA and TBHQ. Figure 8 shows that peroxide values had the smallest increase with TBHQ at 200 ppm stored in darkness. Figure 9 illustrates TBA values with the smallest increase again with TBHQ at 200 ppm stored in darkness. It can also be seen in the figures that increase in storage temperature increased oxidation slightly.

Raghavan and Hultin (2005) reported on the effectiveness of phenolic antioxidants with haddock muscle samples. Figure 10 shows that the use of TBHQ on the haddock muscle samples stored on ice for a period of 12 days resulted in no oxidation and no painty odor (in terms of increased TBARS and sensory analysis, respectively) in comparison with BHA and BHT.

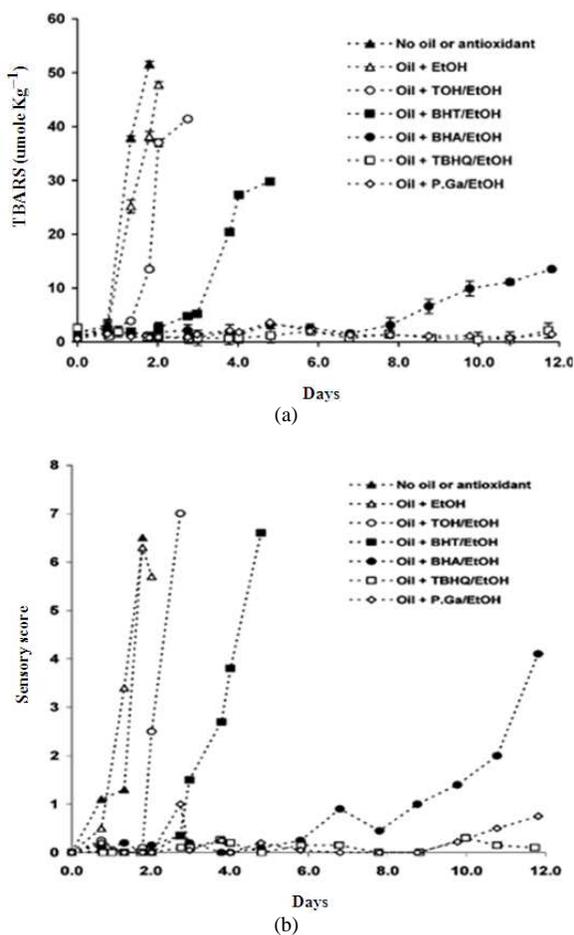


Fig. 10: (a) TBARS values (b) Sensory analysis of painty odor for haddock muscle stored on ice for 12 days (Raghavan and Hultin, 2005)

The effectiveness of the phenolic antioxidants was suggested as being largely influenced by the number of hydroxyl groups available for free radical scavenging. As TBHQ has two hydroxyl groups it gave the best performance over BHA and BHT which have one group.

Ethylenediaminetetraacetic Acid (EDTA):

Ethylenediaminetetraacetic Acid (EDTA) is known as a chelating, sequestering and metal complexing agent (Shelef and Seiter, 2005). EDTA is a member of the polyaminocarboxylic acids group which can be added to fish to remove trace metals through chelation that may act as prooxidants. By removing these catalysts of the free radical mechanism, EDTA can be classified as a lipid oxidation inhibitor. It has, also, shown antimicrobial properties due to the binding of divalent

cations found in bacterial cell walls (Shelef and Seiter, 2005).

As phenolic antioxidants have regulatory limits, they are only added during the grinding stage Shelef and Seiter (2005) used a 1% EDTA solution dip to repress the growth of *Pseudomonas* species. Enhanced lipid oxidation inhibition occurred when the antioxidant mixture was added early in the processing during grinding and then again during washing and prior to storage. While recovering functional proteins from herring by an acid solubilization process, Undeland *et al.* (2005) found that the addition of EDTA (0.044%, 1.5 mM) and erythorbate (0.2%, 9.3 mM) to herring fillets significantly reduced lipid oxidation. Kelleher *et al.* (1992) reported similar results.

Controlling microbial spoilage: Antimicrobial compounds can be found in food naturally formed during processing or added as an ingredient (Ray, 2004). If the antimicrobial is a food additive, it must qualify as being safe according to the specific country's legislation. In the United States, the additive must be GRAS-listed (Generally Recognized as Safe) according to the American Food and Drug Administration (FDA, 2009). In Canada, it must fall under GMP (Good Manufacturing Practice) in accordance with the Canadian Food and Drug Act (HC, 2006). Antimicrobials may be classified as direct or indirect based on the compound's properties and activity. Common antimicrobial compounds include: nitrites, sulfides and organic acids (Chipley, 2005; Ray, 2004; Archer, 2002).

Nitrites: Nitrites are usually added as salts (sodium nitrite or potassium nitrite) along with NaCl, ascorbate and erythorbate to meat, poultry and fish products as an antimicrobial to the toxin producing *Clostridium botulinum* and to enhance color (Ray, 2004). Besides their exceptional antibacterial effect in controlling the anaerobic bacteria *C. botulinum*, *Staphylococcus aureus*, *Yersinia enterocolitica*, nitrite salts are extremely effective in controlling color, odor and lipid oxidation (Sindelar and Houser, 2009; Lovenklev *et al.*, 2004; Archer, 2002; De Giusti and De Vito, 1992; Roberts, 1975). Although its antimicrobial mechanisms are not properly understood, it is known that nitrites may be involved in reactions with enzymes in vegetative cells and germinating spores, restricted use of iron by bacteria, limiting transport due to interference with membrane permeability (Ray, 2004).

It has been shown that the bactericidal nature is not that of nitrite alone but rather compounds derived from

it during food preparation (Cammack *et al.*, 1999). Nitrite antibacterial effect is enhanced at lower pH (5-6) and in the presence of reducing agents such as ascorbate and erythorbate (Ray, 2004). According to the US FDA, current regulatory limit for nitrite is 156 ppm (Ryser and Marth, 1999). In Canada, however, nitrite is limited to 200 ppm in meat and marine mammals but prohibited as a fish additive (DJC, 2009). The cause for concern is associated with the possible formation of carcinogenic nitrosamines with prolonged exposure. However, no epidemiological study has shown a connection between nitrate/nitrite consumption and a specific cancer or cancer risk (Sindelar and Houser, 2009). Nitrosamine formation effects can be negated by addition of sodium ascorbate with nitrate and nitrite salts for the preservation of meat (Rywotycki, 2002; Mirvish *et al.*, 1972).

Sulphites: The use of sulphiting agents to control undesirable microbial growth and activity is one of the oldest applications dating back at least to Roman times. Sulphur dioxide and the salts potassium bisulphite, potassium metabisulphite, sodium bisulphite, sodium metabisulphite and sodium sulphite are listed as GRAS in the US Code of Federal Regulations provided that they are not used in foods which are recognized as major dietary sources of thiamine (Walker, 1985).

Sodium sulfite is used as an antimicrobial agent in soft fruit, fruit juices, wines, beverages, sausages, pickles and fresh shrimp. Sulfides are effective against molds, yeasts and aerobic Gram-negative bacilli. The microbial activity is produced by the undissociated sulfurous acid which enters the cell and reacts with thiol groups of proteins, enzymes and cofactors (Ray, 2004).

Dyett and Shelley (1966) tested the effects of sulphite as preservative for meat sausages. The results indicated that the preservative had a significant inhibition effects on coli-aerogenes and other Gram negative microorganisms at room temperature. Omojowo *et al.* (2009) compared the salts of metabisulphite and sorbate with concentration of 1-3% on the safety and shelf life of smoked catfish. They reported that both are good in reducing the *E. coli* and *Streptococcus* sp. They also found that potassium sorbate had no effect on *Staphylococcus* count throughout the period of storage while only 5% sodium metabisulphite was able to reduce the *Staphylococcus* count to 0 and remained 0 until the end of 8th week storage.

The applications of sulphiting agents to meat and fish products are limited due to the destruction of thiamine (Walker, 1985). In UK, they may be applied to

specified comminuted products such as fresh sausage in which they function primarily as antimicrobial agents, particularly in controlling *Enterobacteriaceae* including pathogenic *Salmonellae* (Banks and Board, 1982). Currently, sulfites are not permitted in Canada as meat or fish additives (DJC, 2009).

Lactic acid/lactates: Lactic acid has shown some antimicrobial properties when used as a rinse for beef, pork and chicken carcasses. This inhibitory capacity is due to the reduction in pH to levels where the bacteria cannot initiate growth (Doores, 2005). De Wit and Rombouts (1990) reported antimicrobial activity of sodium lactate (5%) against various psychrotrophic lactic acid bacteria. Houtsma *et al.* (1993) reported extensively on the minimum inhibitory concentration of sodium lactate under optimal growth conditions (pH 6.5, 20°C) for bacteria isolated from spoiled and unspoiled meat products (Table 10). Tan and Shelef (2002) reported enhanced microbial stability in refrigerated fresh ground pork that was treated with a combination of sodium chloride (1%, w/w) and sodium lactate (2%, w/w).

Sallam (2007) evaluated the shelf life, chemical quality and sensory attributes of salmon slices treated by dipping in 2.5% aqueous solution of sodium Acetate (NaA), sodium Lactate (NaL), or sodium Citrate (NaC) during refrigerated storage at 1°C. He reported significant reduction in K value, Hypoxanthine (Hx) concentration, Total Volatile Base Nitrogen (TVBN) and Trimethylamine (TMA) in treated salmon slices in terms of chemical analyses. A shelf life of 8, 12, 12 and 15 days was estimated for control and salmon treated with NaL, NaC and NaA, respectively.

Lactic acid bacteria are effective in inhabiting undesirable microorganisms in food by producing lactic acid and other organic acids (Matamoros *et al.*, 2009). Matamoros *et al.* (2009) isolated several species of psychrotrophic lactic acid bacteria from various seafood products. Tome *et al.* (2008) succeeded in controlling growth of pathogenic *Listeria monocytogenes* using several lactic acid bacteria on cold-smoked salmon. The lactic acid bacteria strain *Enterococcus faecium* ET05 showed the best controlling results in the study.

Table 10: Minimum inhibitory concentration of sodium lactate (Houtsma *et al.*, 1993)

Bacteria	Minimum Inhibitory Concentration (MIC) (mM)
<i>Salmonella</i> spp.	714-982
<i>Listeria monocytogenes</i> and <i>L. innocua</i>	804-982
<i>Pseudomonas</i> spp. and <i>Yersinia</i> spp.	714-982
<i>Campylobacter</i> spp.	179
<i>S. aureus</i> and <i>Lactobacillus coryniformis</i>	268
<i>Brochothrix</i>	804

Park *et al.* (2005) assessed the antimicrobial effects of lactic acid producing bacteria culture condensate mixture on *Salmonella enteritidis*. At a concentration of 5 and 10% of culture condensate mixture, the growth of *S. enteritidis* was significantly retarded by observing the optical density of broth. However, their ability as inhibitors of pathogenic and spoilage bacteria needs to be investigated in practical use.

Ascorbic acid: Ascorbic acid (vitamin C), sodium ascorbate and D-isoascorbate (erythorbate) have been shown to enhance antimicrobial activity of sulfites and nitrites (Baird-Parker and Baillie, 1974; Raevuori, 1975). The enhanced activity is due to both the antioxidant properties as well as the sequestering of iron (Tompkin *et al.*, 2007). It is commonly known that Practice-listed with meats and fish (DJC, 2009). Ascorbate and its isomer (erythorbate) have antioxidant properties that oxidize reactive oxygen species producing water. Kelleher *et al.* (1992) and Undeland *et al.* (2005) stated that ascorbic acid has proven effective as an antimicrobial at 0.2%.

Raevuori (1975) reported that the addition of 500 mg kg⁻¹ of sodium erythorbate and 200 mg kg⁻¹ sodium nitrite had prevented the growth of *Bacillus cereus* spores in sausages kept at 20°C for 48 h. As previously mentioned, they also may inhibit the formation of carcinogenic nitrosamines which may form the use of nitrite (Mirvish *et al.*, 1972). This finding resulted in the USDA limiting sodium nitrite input to 120 µg g⁻¹ with the addition of 550 µg g⁻¹ of sodium ascorbate or erythorbate (Tompkin, 2005). According to the Canadian Food and Drug Act, ascorbic acid and erythorbic acid are Good Manufacturing Practice-listed with meats and fish (DJC, 2009).

Benzoic acid: Benzoic acid and sodium benzoate are used as a preservative in products that are acidic in nature. Chipley (2005) states that benzoic acid and its salt are generally used to inhibit yeasts and fungi rather than bacteria. The benzoic acid stays as uncharged molecule under low pH conditions which is the form to readily cross the cell membrane. While once in the cytosol, the acid will dissociate because of basically neutral pH environment. The anion and protons released from original molecules cannot diffuse back across the membrane, hence accumulated in the cytosol. As a result, the acidification of the cytosol and the depletion of ATP as energy will cause physiological malfunction and finally inhibition of microorganism growth (Krebs *et al.*, 1983; Warth, 1991; Brul and Coote, 1999; Hazan *et al.*, 2004). The depletion of energy is mainly used in maintaining a lowered intracellular benzoate concentration (Warth, 1988).

However, according to Hazan *et al.* (2004), yeasts such as *Saccharomyces* and *Zygosaccharomyces* have intrinsic ability to resist benzoic acid under the tolerable toxicological limits. The combination of benzoic acid treatment and nitrogen starvation conditions is suggested by the author to enforce effective food preservation from yeast spoilage.

Efluvewewere and Ajiboye (1996) conducted a study on control of microbial spoilage of smoked catfish by different chemical preservatives. They reported that sodium benzoate-treated samples could only be effective for the first 4 days after treatment under tropic environment. Van Spreekens (1977) reported that typical Pseudomonad-like shrimp-spoiling bacteria was inhibited by adding at least 0.8% benzoic acid as a preservative.

Dąbrowski *et al.* (2002) conducted a qualitative and quantitative studies on microflora of low-salt herring slices supplemented with 0, 0.2 and 0.3% of sodium benzoate (E-211). They observed that sodium benzoate reduced diversity of bacteria and yeasts in a tested product and exerted no influence on the total number of bacteria and yeasts. It gave the evidence that an empty ecological niche was created after elimination of some species by the preservative and the remainders substituted them.

In Canada, the limits set on benzoic acid are 1000 ppm for marinated or similarly cold-processed packaged fish and meat. Alternatively, benzoic acid is not permitted for unstandardized meat and fish products (DJC, 2009).

CONCLUSION

The spoilage of fish and fish products depends on a number of factors. These factors as well as the spoilage mechanism must be thoroughly understood before developing proper handling and pretreatment methods and preservation techniques for food products. To stop the rapid spoilage of fish, it is essential to store the fish at 0°C after catching during harvesting. However, the energy intensive freezing operations is only a temporary method for preservation of fish as freeze storage can decrease microbial and enzymatic spoilage but cannot prevent oxidative spoilage. After a comprehensive review of current preservation techniques (which include: water activity; ant enzymatic, antimicrobial and ant oxidative techniques), a process involving the addition of an EDTA/TBHQ combination and ascorbic acid can be the most positive for controlling the spoilage of fish and fish product. The suggested process would address antimicrobial activity as well as destructive oxidation of the desired lipids and

fats. The addition of EDTA (1 mM) and TBHQ (at the regulated level of 0.02% by weight of lipid content) have proven to be effective in limiting lipid oxidation and ascorbic acid has also proven effective as an antimicrobial at 0.2%. Addition of preservative additives (EDTA/TBHQ/ascorbic acid) and storage at refrigerated temperatures (5°C) in darkness could be the best possible way to preserve the fish and fish products. However, more efforts are required to understand the role of proximate composition of fish, post harvest history, environmental conditions, initial microbial load, type and nature of bacteria and their interaction in order to optimize the shelf-life of fish.

ACKNOWLEDGMENT

This research is supported by the National Science and Engineering Council (NSERC) of Canada through a Strategic Research Grant.

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