HIGH PRESSURE PROCESSING OF FRESH TUNA FISH AND ITS EFFECTS ON SHELF LIFE

BY

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A thesis submitted to the Faculty of Graduate Studies and Research in partial Fulfillment of the degree of Master of Science

August 2004

Suggested short title:

High Pressure Processing of Fish

ABSTRACT

Tuna is highly perishable and has been implicated in histamine poisoning because of high histidine levels in the muscle. There is high demand for fresh tuna fillet and steaks for Japanese type foods, and as a grilled item in restaurants. Demand for fresh, additive-free and safe seafood products has stimulated efforts to discover novel methods to prolong the shelf life of fresh products with minimum loss of quality. High pressure processing was investigated for its effects on quality and useful shelf life of fresh tuna. Fresh tuna was subjected to various pressure treatments (220 MPa, 200 MPa, 150 MPa), holding times (30 min, 15 min) and temperature (below 20°C). The pressure treated and control (untreated) samples were analyzed for initial physicochemical properties and subsequent changes during chilled storage. Color parameters (L*, a* and b* values), texture, drip loss, pH, sensory attributes, endoprotease activity, TBA, TVB and histamine levels were all evaluated.

All the pressure-treated samples lost their glossiness, and their redness decreased with pressure and holding time. Pressure treatment increased yellowness of the samples, and the 'b' values increased throughout storage. High pressure processing above 150 MPa resulted in firmer muscles with higher springiness. High pressure also resulted in increased drip loss for all pressure treated samples. However during chilled storage increase in drip loss was significantly faster in untreated samples than in pressure treated samples. Proteolytic activity did not change significantly during storage, unlike the TVB levels that increased during storage. Pressure treatments at 220 MPa/30 or 15 min, and 200 MPa/30 min reduced TVB values the most. None of applied pressure conditions induced major changes in initial pH values. Also no consistent pattern was observed for TBA levels, although the levels were low and indicative of high quality products. Histamine formation was inhibited by pressure treatment at 220 MPa/30 min while other pressure levels appeared to enhance histamine formation. There was no relationship between sensory attributes and histamine content.

A pressure level of 220 MPa/30 min was optimal in controlling proteolysis, texture degradation, as well as histamine and TVB formation, without promoting lipid oxidation in tuna. It also achieved a 9-day shelf life extension of the product.

RESUMÉ

Le thon est fortement périssable et a été impliqué dans l'empoisonnement de l'histamine à cause de niveau très élevé d'histidine dans la chair du poisson. Il y a un forte demande pour le filet de thon frais et de steaks de type japonais de même que les grillades. La demande pour les produits frais de mer, sans additifs et sûrs a stimulé le milieu scientifique afin de découvrir de nouvelles méthodes pour prolonger la durée de vie des produits frais avec la perte minimale de qualité. Le traitement à haute pression a été évalué pour ses effets sur la qualité et la durée de vie du thon frais. Le thon frais a été soumis aux divers traitements de pression (220 MPa, 200 MPa, 150 MPa), avec les temps de rétention de 30 minutes et 15 minutes, et une température sous 20°C. Les échantillons pressurisés et non pressurisés ont été analysés pour leurs propriétés physicochimiques initiales ainsi que les changements associés au stockage. Les paramètres de couleur (CIE L*, a* et b*), la texture, l'exsudat, pH, les attributs sensoriels, l'activité endo-protéase, TBA, TVB et le niveau d'histamine ont été évalués.

Tous les échantillons pressurisés ont perdu leur lustre, et leur rougeur a diminué avec la pression et le temps de rétention. La pressurisation a augmenté le jaune des échantillons et les valeurs de 'b' ont augmentés au cours du stockage. Le traitement à haute pression au-dessus de 150 MPa a augmenté la fermeté des muscles ainsi qu'une élasticité plus élevée. Par le fait même, le traitement à haute pression a causé l'augmentation de l'exsudat de tous les échantillons pressurisés. Cependant, l'exsudat était significativement plus rapide sur les échantillons non traités que sur les échantillons pressurisés. L'activité protéolytique n'a pas changé significativement durant le stockage, à la différence des niveaux de TVB qui ont augmenté durant le stockage. La pressurisation à 220 MPa/30 ou 15 minutes et 200 MPa/30 minutes ont réduit le niveau de TBA de façon plus marqué. Aucune des conditions de pression appliquées n'a provoqué des changements majeurs sur le pH initial. De même qu'aucun modèle cohérent n'a été observé pour le niveau de TBA, bien que les niveaux soient bas et indicatifs de produits de haute qualité. La formation d'histamine a été inhibée suite au traitement à 220 MPa/30 minutes tandis que d'autres niveaux de pression ont semblés augmenter la formation

d'histamine. Il n'y avait aucun rapport entre les attributs sensoriels et le contenu d'histamine.

Un niveau de pression de 220 MPa/30 minutes était optimal pour contrôler la protéolyse, la dégradation de la texture, ainsi que l'histamine et la formation de TVB, sans promouvoir l'oxydation des lipides du thon. Par la même occasion, une durée de vie du produit de 9 jours a été atteint.

ACKNOLEDGEMENTS

I would like to express my profound gratitude to my supervisor Dr. Benjamin. K. Simpson for his invaluable advice, sincere guidance, great support and assistance. He supervised my work with great patience and constantly encouraged me during my study at the Macdonald campus of McGill University.

I wish to humbly express my gratitude to my co-supervisor Dr. H. Ramaswamy for his sincere guidance, great advice and support during my study. Also I would like to express my appreciation to Dr. Ramaswamy for making the high pressure and other laboratory equipments available to me.

My special thanks go to Dr. Gopal from India, who I had the opportunity to work and cooperate with, for his valuable advice and assistance during his visit to the Macdonald campus.

I also would like to express my appreciation to my fellow college students in both Dr. Ramaswamy's and Dr. Simpson's labs; Ms. Jing Hong, Mr. Yanwen Shao, Ms. Neda Maftunazad, Mr. Heping Li, Mr. Martin Macouzet, Ms. Sahel Soltani, Ms. Thi Thuy Duong Nguyen and Mr. Santosh Gunjal, Ms Alberta Aryee as well as my friends and staff in the Food Science Department for their valuable friendship.

My heartfelt thanks go to my husband Dr. Alireza Shooshtari for his tremendous support, sacrifices and constant encouragement, and also my dear daughter Dorsa, for being an incredible source of hope and energy for me and finally, to my wonderful parents and siblings for their great love, huge support and compassion.

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ABBREVIATIONS

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ASP	Amnesic shellfish poisoning
ATP	Adenosine triphosphate
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BSA	Bovine serum albumin
CA	Controlled atmosphere
CEE	Comunidades eroupeas
CSW	Chilled seawater
CTC	Chlortetracycline
DSP	Diarrheic shellfish poisoning
DNA	Deoxy nucleic acid
EDTA	Ethylene diamine tetra acetic acid
ESP	Expressed soluble proteins
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration
GRAS	Generally regarded as safe
GOX	Glucose oxidase
HACCP	Hazard analysis critical control point
HP	High pressure
HX	Hypoxanthine
IMP	Inosine monophosphate
INO	Inosine
INQ	Index of nutritional quality
IPK	Instantaneous pressure kill
IAEA	International atomic energy agency
KS	Potassium sorbate
MA	Modified atmosphare

MAP	Modified atmosphere packaging
MPa	Mega Pascal
MRSW	Modified refrigerated seawater
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
NDGA	Nordihydroguaiaretic acid
NSP	Neurotoxic shellfish poisoning
OPT	O-Phthaldialdehyde
OTC	Oxytetracycline
PG	Propyl gallate
PME	Pectin methyl esterase
PPO	Polyphenoloxidase
PSP	Paralytic shellfish poisoning
RSW	Refrigerated sea water
SAS	Statistical analysis system
TBA	Thiobarbituric acid
TBHQ	Tertiary butylhydroquinone
THBP	Trihydroxybuterophenone
TMAO	Trimethylamine oxide
TMA	Trimethylamine
TVB	Total volatile base
WHC	Water holding capacity
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

Freshly caught fish is a favorable and delicate food due to its soft and moist texture, mild sweat flavor, and great nutrition and health value. However, because of its high moisture and free amino acid content combined with lower levels of connective tissue compared with other muscle foods, seafoods are more susceptible to spoilage and therefore have a short shelf-life. Hence it is essential to take immediate steps to assure that the fresh quality and safety of fish products are well preserved. Seafood quality deterioration occurs from wide range of causes. It can start from poor catching practices, poor handling and keeping of raw material on board ships, sub-standard manufacturing practices and also inefficient refrigeration and storage (Alasalvar and Taylor, 2002). When fresh fish deteriorates, it passes from rigor mortis to autolysis, and finally to bacterial spoilage. Loss of freshness is caused by endogenous biochemical changes in muscle, not only by bacterial action, but also through natural enzymes degradation - as it has been shown that fish will spoil even under aseptic conditions (Satio et al., 1959). To ensure that fresh quality is well preserved, and to establish the most efficient methods for preservation of seafood quality, both objective and subjective methods of quality analysis should be performed (Alasalvar and Taylor, 2002). Chemical, biochemical, physical and microbiological methods have been used as objective methods to evaluate the quality of fish during chilled storage. The purpose of subjective evaluations is to make sure that those results show good agreement with the objective tests (Alasalvar and Taylor, 2002).

In many countries fish is caught far from the coast and by the time fish arrives to the market it has been kept for several days in ice, and thus would have a limited commercial shelf life. Therefore in order to retail, it is essential to prolong the shelf life of fresh seafood products as much as possible. Due to limitations of current methods for producing fresh, additive free and safe seafood products, investigation and application of novel physical treatments such as high pressure have been developed in recent years, which has offered great advantages over conventional methods. High pressure (HP) has been used to extend the average storage life of various kinds of foods. In Japan high

pressure technology has been widely applied for small scales production of fruit-based products such as jams and juices. Also in France (orange juice) and USA (avocado spread) some pressurized foods has been produced for the market. So far however not many of experimental results of pressurized food products, specially in muscle food, have been transferred to industry (Hurtado et al., 2000).

It is known that high pressure processing alter the structure and function of many proteins and therefore affects the properties of meat products. High pressure effects on muscle food strongly depend on both the technological processing factors and nature of the meat system (Carballo et al., 1999). The level, progression, and time of pressure treatment and temperature conditions significantly influence properties of meat systems (Carballo et al., 1999). For example pressurization of raw fish muscle at over 200 MPa, induces a whitish tone in the fish flesh similar to that produced by cooking, and this phenomenon increases with pressure (Ohshima et al., 1993). High pressure has been applied in the tenderization of meat and for the preservation of many muscle foods such as chicken, pork, salmon, surimi gel products, trout, shrimp, turbot, hake, etc (Suzuki et al., 1992; Knorr, 1993; Ohshima et al., 1993; O'Brien and Marshall, 1996; Houjaij, 2000; Chevalier et al., 2001; Hurtado et al., 2003). HP has shown positive effects on some properties of these products such as texture, taste, flavor and enzyme inactivation and also on shelf life extension.

Besides quality and freshness, safety of fish used for consumption is an important issue which should not be ignored. The huge interest in seafood consumption in the United States, Japan and several other countries, as well as the current tendency for consumption of various forms of fresh and raw fish/shellfish products, has made public health safety of seafood a more pressing issue. Hence, careful assessment of seafood safety for consumption along with analysis for fresh quality is an imperative task. There are many food-borne intoxications and infections related to fish and shellfish products (Table 1.1). Histamine poisoning has been one of the three most frequently reported illnesses associated with seafood in the USA (FAO, 1994). Histamine poisoning is due to consumption of fish from scombroid species such as tuna, bonito and mackerel that have

high amounts of histidine in their muscles (Taylor, 1989). Histamine formation is induced by high temperature abuse during post harvest, handling and storage. Histamine producing bacteria possessing histidine decarboxylase convert histidine to histamine (Rawles et al., 1996). Therefore, in the present research on 'high pressure processing of fresh tuna fish and its effects on shelf-life extension', histamine content of samples was measured as an index of the quality and safety of tuna fish.

Table 1- Fish-borne intoxication and infections (FAO, 1994)

Types of fish-borne illnesses			
Type of illness		Causative agent	
Infections	**Infections**	***Listeria monocytogenes, Salmonella sp., Shigella sp.***	
	Toxic-infections	***Vibrio paraheamolyticus, V. cholerae, E. coli, Salmonella sp.***	
Intoxications	**Chemical**	**Heavy metals: *Hg, Cd, Pb*. Dioxines and PCBs. Additives: *nitrites, sulfites***	
	Biotoxins	**Ciguatera, Paralytic shellfish poisoning (PSP), Diarrheic shelfish poisoning (DSP), Amnesic shellfish poisoning (ASP), Neurotoxic shellfish poisoning (NSP), Histamine**	
	Microbial	**Bacterial**	***Staphylococcus aureus, Clostridium botulinum***
		Viral	**Hepatite type A, Norwalk, Calicivirus, Non-A and Non-B**
		Parasitic	**Nematodes (round worms), Cestodes (tape worms), Trematodes (flukes)**

The project objectives were:

1- To study the influence of pressure levels between 150 MPa to 220 MPa with two pressure holding times (15 min and 30 min), at a moderate temperature of about 20°C, on several factors associated with quality, appearance, safety and shelf life of tuna fish, both immediately after treatment as well as storage for various times at 4 ± 1°C. The specific quality related factors were:

- *Physical attributes of tuna fillet such as color, texture, drip loss*
- Biochemical properties such as pH, total volatile base nitrogen (TVB), lipid oxidation (TBA), and histamine formation
- Autolytic
- Sensory evaluation

2- To determine the optimal pressure treatment levels for maintaining freshness of tuna meat for commercial purpose.

3- To carry out statistical analysis of experimental results (ANOVA, SAS/STAT) for testing the significance of results.

CHAPTER 2

LITERATURE REVIEIW

2.1 Fish as Food

Seafood is considered as an excellent source of nutrients for human health and is appreciated world wide for their distinct flavors (Shahidi and Cadwallader, 1997). Seafood is especially favorable because it contains long chain omega-3 polyunsaturated fatty acids, whose health benefits have been greatly touted in recent studies. It is believed that particularly omega-3 (w) and omega-6 (w) series of fatty acids play an important role in non-energy producing biological functions and the lack of these components in human diet can cause a variety of diseases such as cardiovascular, hypertension, inflammatory and autoimmune disorders, depression and certain disrupted neurological functions. Fish is very important in human diet and it can help to fulfill three out of ten popular suggestions for rational nutrition. It provides protein of high biological value, a large variety of minerals (Ca, Fe, P) as well as microelements (I_2) and vitamins (Pedrosa-Menabroti and Regenstein, 1988). Fish protein contains all the essential amino acids and its biological value is similar to those of milk, eggs and mammalian meat proteins (Table 2.1). Fish proteins are also known to be highly digestible with a protein efficiency ratio ranging from 2.7 to 3.2 (Javaheri et al, 1984). The vitamin content of fish is similar to that of mammals except in the case of the A and D vitamins, which are higher in fatty fish species and abundant in the liver of some species such as halibut and cod (Sareevoravitkul, 1995).

Rising demands for seafood has led to higher production in seafood industry. In Canada alone aquaculture production reached up to 127,237 tons in 2001, worth $600.4 million, and the total landings from Atlantic and Pacific coasts of Canada was estimated at about 990,670 tons with a landed value of $ 2.00 billion (Agri-food Canada, 2000).

Table 2.1- Essential amino acids in various protein foods (%)

Huss (1988)

Amino acid	Fish	Milk	Beef	Eggs
Lysine	8.8	8.1	9.3	6.8
Tryptophan	1.0	1.6	1.1	1.9
Histidine	2.0	2.6	3.8	2.2
Phenylalanine	3.9	5.3	4.5	5.4
Leucine	8.4	10.2	8.2	8.4
Isoleucine	6.0	7.2	5.2	7.1
Threonine	4.6	4.4	4.2	5.5
Methionine-cysteine	4.0	4.3	2.9	3.3
Valine	6.0	7.6	5.0	8.1

2.2 Deterioration of Fish

Fish deterioration is often thought of as simply the development of bad odor and bad flavor or any change that decreases palatability and/or quality of fish or shellfish (Singleton and Sainsbury, 1978). Fish spoilage is usually due to microbial spoilage, autolytic enzyme activity, unfavorable chemical changes, mainly trimethylamine oxide (TMAO) breakdown, lipid oxidation, lipid hydrolysis and protein denaturation (Hall, 1997). The other causes of quality loss such as physical damage during handling, dehydration and contamination can also be observed. Any of these will predominate in

any given food; therefore to control quality loss will depend on several factors, including the chemical composition of the fish and shellfish (Ashie et al., 1996). The deterioration happens in two overlapping phases; first autolysis, can be regarded as loss of freshness, a deterioration consequent upon destruction of balance of the fish's postmortem metabolic functions, followed by bacterial action that produces more pronounced effects of obvious spoilage which eventually causes fish meat to become inedible (Houjaij, 2000).

2.2.1 Microbial Spoilage

Fish of any species generally go through microbial spoilage in much same way, but there are wide differences in the way that fish of different families, and even different species in the family, deteriorate (Connell, 1990). The major biological molecules in fish (i.e., carbohydrates, proteins, and lipids) all serve as substrates for microorganisms present in the gills, gut, and skin which along with endogenous enzymes metabolize compounds causing off-flavor, texture deterioration, discoloration and other adverse changes in fish muscle (Ashie et al., 1996). Microorganisms differ in their biochemical activities. Some ferment carbohydrates to generate acid and/or gas, others degrade proteins to release ammonia and other amino compounds; some others convert nitrites into nitrogen or its oxides. Microbial spoilage therefore, may vary from gross and stinking decay, mushiness, to acid and/or gas production. In addition, certain toxin-producing bacteria may release their toxins into food to render them harmful for human consumption (Ashie et al., 1996). The method of capture, location of fishing grounds, season of the year, fat content and fish size all influence the rate of microbial spoilage of fish. Lean fish keeps well in ice for about 12-18 days, after which it is likely to become inedible, or nearly so (Connell, 1990). Fish from cold-water regions have a larger proportion of psychrotrophs among their natural micro flora, which can shorten the chill shelf-life appreciably (Borresen and Storm, 1993). These fishes are preinoculated with psychrotrophic Gram-negative spoilage bacteria and such bacteria would decrease the lag time before outgrowth and proliferation during storage (Ashie et al., 1996). Aerobically refrigerated fish are mainly spoiled by (1) endogenous microorganisms which are aerobic

psychrotrophic microorganisms, e.g., *Pseudomonas*, *Alteromonas*, and to a smaller extent, *Vibrio, Achromabacter, Acetinomonas*, and *Flavobacterium* and (2) contamination from exterior sources such as handling, processing equipment, etc. (Houjaij, 2000). Trimethylamine (TMA) produced by reduction of trimethylamine oxide (TMAO), by either aoutolytic processes or by certain bacteria, plays a role in the off-flavor and odors in postmortem fish muscle (Pedrosa-Menabrito and Regenstein, 1987). TMAO reduction is mainly associated with *Alteromonas, Photobacterium, Aeromonas, Vibrio, S. Putrefactions,* and intestinal bacteria of the *Enterobacteriaceae* group (Kruk and Lee, 1982). Off-odor/off-flavor compounds produced by bacterial spoilage are listed in Table 2.2 (FAO, 1995).

Spoilage microorganisms are also implicated in the production of extracellular proteinase required for enzymatic degradation of exogenous proteins to peptides and amino acids for cellular uptake (Lee and Chang, 1990). Serine proteinases, acid proteinases, (cathepsin D, pepsin), metallo proteinases (carboxypeptidases, collagenases) and cysteine proteinases (cathepsins B, L and H) are among these extracellular proteinases (Al-Omirah, 1996).

Table 2.2- Substrate and off-odor/off-flavor compounds produced by bacteria during spoilage of fish

Substrate	Compounds produced by bacterial activity
TMAO	**TMA**
Cysteine	**H_2S**
Methionine	**CH_3SH_2 $(CH3)_2S$**
carbohydrates and lactate	**acetates, CO_2, H_2O**
inosine, IMP	**hypoxanthine**
amino-acids, urea	**esters, ketones, aldehydes, NH_3**

2.2.2 Enzymatic Spoilage

Studies have shown that the shelf life of sterile fresh fish is similar to that of normal fish, and although spoilage of seafoods is invariably attributed to contamination micro-organisms, exclusion of bacteria does not change the rate at which the product becomes unacceptable to sensory panelists (Fletcher and Satham, 1988). The autolysis reaction of endogenous enzymes, from both fish gut and muscle tissues synthesized and secreted into the extracellular matrix promotes autolysis in postmortem seafood. Proteolysis, glycolysis, nucleic acid (ATP) breakdown and lipid hydrolysis/oxidation are the major autolytic reactions causing deterioration of fresh seafood quality (Ashie et al, 1996).

Endogenous enzymes including those participating in the muscle contraction remain invariably active and continue to function in the postmortem fish (Hobbs, 1982). In the postmortem animal ATP (the main carrier of energy in cell metabolism) is gradually depleted and not restored by glycogen, leading to permanent actomysin crosslinkages and subsequently the stiffening associated with rigor mortis. Rigor mortis sets in when ATP concentration falls below a critical level (Pedrosa-Menabrito et al., 1987).

After capture, fish struggles, and in the reduced oxygen milieu, the glycogen remaining in muscle cells breaks down to glucose and lactic acid (glycolysis), similar to that which occurs in mammalian meats. Lactic acid buildup by post mortem glycolysis causes an acidic pH (6.2 - 6.5) in muscle tissue from the physiological range. Values below pH 6.0 have been detected in some species such as halibat, mackerel, sturgeon and tuna (Izquierdo-Pulido, 1992). At lower pH levels, usually proteins approach their iso-electric points causing a decrease in water holding capacity (WHC), due to protein-protein interactions. Drip-loss or moisture exudation results in a chalky, tough, and dry texture (Pedrosa-Menabrito and Regenstein, 1988). Because the level of glycogen in fish muscles is low compared with mammalian muscle, consequently the final post mortem pH is higher in fish muscles, which causes fish meat to be more susceptible to microbial growth (Houjaij, 2000). Continuing activity of endogenous enzymes leads to ATP break

down by a series of rapid dephosphorylation and deamination reactions to adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), followed by a slower stage of degradation of IMP to inosine, hypoxanthine (Hx), xanthine, and finally uric acid (Fig. 2.1) (Pedrosa-Menabrito et al., 1987). In most fish species, a rate determining step exits between xanthine and uric acid; as a result, inosine (INO) and hypoxanthine (Hx) decomposed compounds is defined as the K value, which is an effective index of fish freshness (Oshima et al., 1993):

$$\mathbf{K\ (\%) = \frac{INO + Hx}{ATP + ADP + AMP + IMP + INO + Hx}}$$

During rigor, autolytic changes of fish proceeds further. Proteolytic endoenzymes of fish muscle degrade peptides and proteins causing a softening of the flesh after muscle glycogen are depleted. This condition favors the growth of spoilage microorganisms (Sareevoravitkul, 1995). Fish muscle proteins are degraded by endogenous proteases resulting in quality defects such as "belly burst" in fish species like capelin, herring, and mackerel (Haard et al., 1985). Endogenous proteases also have been implicated in salmon flesh softening (Konagaya, 1985), in the deterioration of connective tissue matrix of cod muscle leading to losses in surimi yield, and in ice-stored and cooked prawn causing mushiness (Linder et al., 1988).

Besides proteolysis, lipid oxidation is a major post harvest deterioration event in fish. Lipids in fish undergo one of two changes; lipolysis and oxidation. Lipid oxidation is catalyzed by the lipoxygenase enzyme present under the fish skin. Postmortem lipolysis proceeds mainly due to enzyme hydrolysis by lipases which may be brought about by microbial or endogenous lipase (Pedrosa-Menabrito, 1988). Lipid hydrolysis leads to production of compounds such as free fatty acids, lysophospholipids, glycophosphocholine and phosphoric acid. Phospholipids are most rapidly hydrolyzed followed by triglycerols, cholesterol esters and wax esters (Sikorski, 1990; Oshima et al., 1993)

Figure 2.1- Degradation of ATP in fish muscle (Eskin, 1990). The enzymes numbered are: (1)ATPase; (2) Myokinase; (3) AMP deaminase; (4) Nucleoside phosphorylase (5) Inosine nucleosidase; (6) Xanthine oxidase.

2.2.3 Chemical spoilage

Chemical spoilage of fish basically includes moisture loss, loss of vitamins and volatile flavors, oxidative rancidity, changes in odor and flavor of fish (Lyver, 1997). Besides enzymatic formation of hydroperoxide, the other types of spoilage in fish are; non-enzymatic browning and hydrolytic rancidity.

In fatty fish which contain high levels of unsaturated fatty acids, rancidity is the major cause of flavor, color and texture deterioration (Hultin et al., 1982). Oxidative rancidity is a chemical process which involves oxidation of unsaturated fatty acid or triglycerides in seafoods via free radical formation, which is illustrated in Fig. 2.2 (Ashie et al., 1996). Several factors, such as degree of unsaturation, temperature, light and water activity and pH, determine the rate of lipid oxidation (Tsuchiya, 1961).

Initiation: $RH \rightarrow R^* + H^*$

$RH + O_2 \rightarrow ROO^* + H^*$

Propagation: $R^* + O_2 \rightarrow ROO^*$

$ROO^* + RH \rightarrow ROOH + R^*$

Termination: $ROO^* + R^* \rightarrow ROOR$

$R^* + R^* \rightarrow R\text{-}R$

$ROO^* + ROO^* \rightarrow ROOR + O_2$

Figure 2.2- Free radical mechanism of unsaturated fatty acid oxidation in seafood (Ashie et al., 1996)

Also some substances in fish muscle known as pro-oxidants can accelerate the oxidation process (Tsuchiya, 1961). Among these biochemicals, substrates such as amino acids, heme compounds, organic acids, and pigments have been shown to catalyze the oxidation reaction either alone or in association with certain trace metal ions. Cu^{2+}, Fe^{2+} and Fe^{3+} are the most important metal ions involved in lipid oxidation (Khayat and Schwall, 1983). Another major chemical deterioration in fish is non-enzymatic browning reactions which lead to discoloration of fish muscles via two types of reactions. One reaction takes place between autooxidative lipids and proteins, and the other (Maillard reaction) occurs between sugars like ribose and amino acids (El-Zeany et al., 1975). The products from autooxidative lipid reaction with protein is due to ionic condensation of primary amino groups of protein with conjugated unsaturated aldehydes of similar active lipid oxidation products resulting from cleavage of unsaturated hydroperoxides (Ashie et al., 1996).

2.3 Fish Safety

Intoxication associated with seafood is the biggest food poisoning outbreak in many countries such as USA, England and Wales (Alasavar and Taylor, 2002). From 1991 to 1997, a total of 78 seafood-borne illness outbreaks resulting in 169 cases were recorded in Canada. The top three reasons for the total number of cases were from histamine, decomposed product and paralytic shellfish poisoning, together representing 60 percent of all cases (FAO , 2000) (Table 2.3). Scombroid poisoning results from ingestion of foods containing high levels of biogenic amines typically histamine, and is one of the most frequently reported illnesses associated with seafood consumption, mainly from scombroid species such as tuna and mackerel. Hence many researchers have suggested the use of these compounds as indicators of seafood safety and quality (Rossi et al., 2002).

Table-2.3 Summary of the number of seafood-borne illness outbreaks and cases in Canada by Causative agent, 1991 to 1997 (FAO, 1999)

Causative agent	**Outbreaks**	**Cases**
Histamine	19	55
Fecal coliforms	15	15
Decomposed fish products	14	26
Paralytic shellfish poisoning	6	21
Ciguatera toxin	6	14
Diarrheic shellfish poisoning	4	4
Staphylococcus aureus	2	2
High bacteria count	2	2
Escherichia coli	2	3
Vibrio parahaemolyticus	2	2
Tetramine	1	2
Salmonella	1	12
E. coli and Salmonella	1	1
Salmonella and S. aureus	1	4
Allergy to undeclared ingredient	1	1
Parasites	1	5
Total	**78**	**169**

2.4 Histamine Scombroid

Histamine scombroid or histamine poisoning is one of commonest food poisoning caused by consumption of fish from the scombroid family such as tuna, mackerel and bonito (Taylor et al., 1986). The exact mechanism of scombrotoxicity is still uncertain, although the toxin is believed to consist of histamine, and possibly putrescine and cadaverine which enhance the toxicity of histamine (Taylor and Sumner, 1986). Enzymatic decarboxylation of histidine (found in abundance in the free form in dark-fleshed fish) in optimal temperature of 20-30°C, results in histamine production. Putrescine and cadaverine are also formed by the decarboxylation of ornithine and lysine, respectively (Farn and Sims, 1986; Taylor and Sumner, 1986). Microbial activity of specific microbial strain is known for producing the enzyme histidine decarboxylase which converts the amino acid histidine to histamine (Rawles et al., 1996). Various kinds of bacteria have been found to have histidine decarboxylase activity, but only a few of them implicated in producing histamine in toxicological levels (Taylor et al., 1979). Microorganisms, specifically *Proteus morganiiby,* and to a lesser degree *Klebsiella pneumoniae*, *Morganella morganii*, and *Hafnia alve*, have been reported to be involved in producing significant levels of histamine in fish (Taylor et al., 1979; Kawabata et al., 1956; Sakabe, 1973; Ferenick, 1970; Havelka, 1967). Usually time/temperature abuse of fish products, from inadequate cooling facilities in fisheries, causes bacterial activation and histidine decarboxylase release in fish tissue. Histamine production can be fairly rapid e.g., in one outbreak after only 3 - 4 hours of storage at room temperature toxin levels reached to maximum level (Kow-Tong and Malison, 1987). Once the enzyme is produced by bacteria, it can continue to produce histamine in fish even in the absence of bacteria. This enzyme can remain or stay in inactive form at temperatures near to frozen temperature. Therefore improper handling of fish can cause rapid intoxication after thawing of fish.

Studies on the production of histamine in mackerel (Murray et al., 1982) showed that fish which were allowed to spoil in ice, had histamine levels that rarely exceeded 5mg/100g of fish, even when it became unfit to eat. However, storage at higher temperatures (especially above 10°C) resulted in high levels of histamine, and production

was shown to be exponential. Therefore, levels of histamine over 5mg/100g of fish indicate that the fish has been unnecessarily exposed to high temperatures. In most cases, the histamine level in illness-causing fish has been above 200 ppm and often 550 ppm. In mahi mahi, histamine accumulation reached to highest level (250mg/100g) at 32°C within 24 h, when incubated at 0-32°C (Baranowski et al., 1990). Also in Kahawai fish, the highest rate of histamine formation and accumulation accrued at 25°C, 330mg/100g muscle within 2 days (Flechter et al., 1995).

Histamine is the only biogenic amine for which the use of maximum level has been established for fish. Though there are indications that fish decomposition can result in production of putrescine and cadaverine, which have the potential to cause illness even in the absence of histamine (Alasavar and Taylor, 2002). Hazard analysis critical control point (HACCP) has mandated monitoring of the histamine and other biogenic amines as safety indicators (Du et al., 2002). The European Union has set up 100 ppm as a maximum average level of histamine for Scombroid and Scomberesocidae families (CEE 1991). While the Food and Drug Administration (FDA) established a level of 50 ppm histamine as an indication of potential health risk in fish (FDA, 1996)

2.5 Fresh Seafood Preservation Methods

Several methods have been used over the years for preservation of fresh fish that can be divided to following groups:

2.5.1 Chilling

Temperature control is the prime factor affecting the speed at which fish spoil (Hall, 1997). Temperature influences the rate of food spoilage by its effects on both enzymatic and microbial activity. The growth of many microorganisms are reduced at temperatures below 10°C and even cold-tolerant bacteria have much longer phase and generation times at temperature near 0°C (Huss, 1988). The most effective method of preserving fresh fish is by chilling to about 0-1°C (Pedersa-Menabrito and Regenstein, 1988). Basically, there are several different conditions for chilling, such as chilling in melting ice, chilling in refrigerated sea water (RSW) and chilling in ice slurry. In chilling in melting ice, media such as block ice, crushed iced, and flake ice are used in fisheries. The average storage shelf life of fish kept at 0°C is between one and two weeks (Pederosa-Menabrito and Regenstein, 1988). Superchilling at subzero temperature or partial freezing involves the storage of fish at -3°C to -4°C, but the disadvantage of this system is the enhanced phospholipid hydrolysis and denaturation of myofibrillar proteins which occur with corresponding decrease in temperature to -5°C (Toyomizu et al., 1981). Ice slurry is made up of sea water and ice (chilled sea water (CSW)), generally in the ratio of 1:2 v/w with a temperature about -1.5°C. The heat transfer between ice and sea water is by convection, thus the rate of chilling is higher than in ice, and by agitation of sea water and ice in a tank the temperature remains constant all over the container (Sikorski, 1990). Unlike chilling in melted ice, in this method unless there is some loss of scales, no harmful effects occur to fish texture. RSW and CO_2-modified refrigerated seawater (MRSW) offer similar advantage as CSW. However great capital investment for requiring corrosion-proof equipment, operating cost, requirement for skilled personnel and potential for psychrophilic bacteria growth, limit application of this method. Also

some species are susceptible to loss of appearance due to bleaching. Furthermore, the uptake of salt may alter the flavor and decrease the market value of fish (Sikorski, 1990). Usually in order to delay fish spoilage and extend the keeping quality, chilling has been employed in combination with other barriers such as modified atmosphere packaging, chemical treatments, high pressure, use of acidulants, antioxidants, etc.

2.5.2 Freezing

About 75% of the weight of fish is water which contains dissolved and colloidal substrates. These substrates reduce the freezing point of fish to below 0°C (-1 to -2°C). In freezing process water converts to ice and concentration of dissolved organic and inorganic salt increases, however the bound water never freezes. Generally, freezing preserves fish by reducing the reaction rate and water activity, hence preventing bacterial growth and autolytic activity (Hall, 1997). The main disadvantage of freezing of fish is protein denaturation due to unavailability of water which causes loss in water holding capacity and drip loss of fish after thawing. To minimize the drip loss, the time spent in the temperature zone of protein denaturation (-1 to -2°C) should be reduced. Therefore quick freezing of fish is a recommended process (Hall, 1997).

2.5.3 Curing (drying, salting and smoking)

In curing, a longer shelf life is achieved by changing the environment of product to an undesirable condition for micro-organisms propagation by increasing the concentration of soluble substrates in the medium. Concentration is increased either by water abstraction (drying), by causing soluble substrates to diffuse in (salting, brining or sugar curing), or by depositing bacteriostatic chemicals like nitrites, aldehydes and phenols in the system (smoking) (Hall, 1997).

2.5.4 Modified Atmospheres

Modified atmosphere (MA) and controlled atmosphere (CA) systems alter the concentration of gases surrounding the products before storage by using varying concentration of carbon dioxide (CO_2), sulfur dioxide (SO_2), oxygen (O_2), carbon monoxide (CO) and nitrogen (N_2) (Lyver, 1997). Carbon dioxide inhibits bacterial and mould activity. Nitrogen acts as an inert atmosphere and prevents rancidity, mould growth and onset of attack by displacing oxygen. During refrigerated storage in CO_2 atmosphere, *Pseudomonas* spp. are totally inhibited or some die, eventually gram-positive organisms mainly *Lactobacillus* spp and *Alteromonas*, predominate in the microflora of the fish which under refrigeration and MA may produce metabolites other than the typical spoilage gram-negative microflora. This in turn may lead to changes in sensorial characteristics of the fish (Houjaij, 2000). Some disadvantages associated with MA/CA systems are; undesirable color changes in some muscle foods and favorable storage conditions which create an anaerobic environment conductive for *Clostridium botulinum* (Sikorski, 1990). Also MA treatment has been shown to increase drip loss (due to dissolution of CO_2 into the surface of muscle and reducing of pH), darkening of red muscle, and rapid conversion of inosine to hypoxanthine (Davies, 1997).

2.5.5 Irradiation/Radiation

In the early 1940s, ionizing radiation method was used in order to inhibit bacterial growth. The primary mechanism of microbial inhibition by ionizing radiation is the breakage of chemical bonds within the DNA molecules, or alteration of membranes permeability and other cellular function (Lopez-Gonzales et al., 1990; Urbain, 1986). Application of irradiation, i.e., low dose irradiation, below the level of 10 kGy, regarded by the joint FAO/IAEA/WHO Expert Committee on Food Irradiation as presenting no toxicological hazard, has been suggested for extending the shelf life of some refrigerated seafood products (Sikorski, 1990). Low-dose irradiation, e.g., 1.5 kGy has been shown not to extend the sensory quality of fish in ice. In contrast, the application of higher doses

of irradiation, e.g., 5-10 kGy, has its own disadvantage such as development of off-flavor in some products (Ehlermann and Reinacher, 1978). It has been known that irradiation can affect microflora and gram-negative non-sporing bacteria exhibit the highest sensitively to ionizing radiation. The resistance of microorganisms to radiation depends on the species and strain and is affected by the stage of development of the population as well as by the properties of the medium and by the condition of irradiation (Houjaij, 2000). Ouattara et al., (2000) studied the combined effect of low-dose gamma irradiation and antimicrobial coating on the shelf life of pre-cooked shrimp (*Penaeus* spp.). Their results showed a significant additive interaction effect of gamma irradiation and antimicrobial coating in reducing the growth of bacteria in pre-cooked peeled shrimp. This effect was characterized by longer lag period, lower growth rates, with a resulting significant shelf life extension in irradiated samples. From this study, changes in appearance, odor and taste as affected by gamma irradiation were not detected by the sensory assessment of panels.

2.5.6 Chemical Treatments and Enzyme Inhibitors

In this method, several preservatives or antioxidants are used. Many chemical components such as boric acid, salicylic acid and many others have been tested for their effectiveness in controlling fish spoilage (Tarr, 1961). They were applied in the form of dips and as additives to ice used for chilling fish (Sareevoravitkul, 1995). Studies have shown that by dipping the fillets in a 1% solution of ethylene diamine tetra acetic acid salts (EDTA), shelf life of iced fish fillet can be extended by a few days (Power et al., 1968; Boyd and Southcott, 1986). This study suggested that the effect was probably caused by inhibition of several groups of psychotropic bacteria, mainly *Pseudomonas* spp. Field et al., (1986), and Lee (1990), found that dipping of the fish in glucose oxidase (GOX)/Glucose solution (1 enzyme unit/ml and 4% glucose) was able to extend the keeping quality of fish. Potassium sorbate (KS), the potassium salt of sorbic acid, is a generally regarded as safe (GRAS) substance used as a food preservative (Sareevoravitkul, 1995). The activity of KS is found with the un-dissociated form of KS,

i.e., the latter form has 10 to 600 time higher antibacterial effect than dissociated molecules (Sikorski, 1990). Some compounds were not suitable for commercial application and have been questioned for their toxicity and high cost, for example sodium nitrite which may cause the formation of toxic and carcinogenic N-nitrosamines. Sodium nitrite has been found to be effective in retarding spoilage of chilled fish and its use is allowed in Canada in amount not exceeding 200 mg/g of the finished product (Tarr, 1961). Also commercial application of antibiotics like chlorotetracycline (CTC) and oxytetracyclin (OTC) have been prevented by public health consideration in US and no longer is used due to concern for the increasing resistance of pathogen, as well as the cost and difficulties involved in determining these compounds (Sareevoravitkul, 1995).

In fish muscle, especially fatty fish, lipid oxidation is the foremost cause of quality deterioration. Therefore, antioxidants are employed in combating lipid oxidation, responsible for off-flavor and off-odor production by interrupting the oxidation reaction, a process called sacrificial oxidation (Fig 2.3). Antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), and propyl gallate (PG) are phenolic compounds, which have been used widely in food materials. Studies by Eun et al., (1993) suggested that BHT, BHA, PG, trihydroxybuterophenone (THBP), and nordihydroguaiaretic acid (NDGA) were able to inhibit oxidation in catfish muscle microsome. Also they found that ascorbic acid and erythorbic acid at low concentration smaller than 200 ppm can act as pro-oxidant which stimulate lipid oxidation in catfish microsome. It is suggested that pro-oxidant properties of ascorbic acid is from the altering of reduction-oxidation potential to reduce metal ions which are natural pro-oxidants (non-heme iron) found in fish muscle into active form (Eun et al., 1993; Kelleher et al., 1992). While the antioxidant property of ascorbic acid at high concentration is due to its behavior as reducing agent toward inactive heme iron which is another natural pro-oxidant in fish and free radical terminator in lipid oxidation (Kelleher et al., 1992). Chelating agents EDTA can completely inhibit lipid oxidation in NADPH-dependent system and non-enzymatic system from flounder muscle (Hultin et al., 1982). Pro-oxidants such as free ionic or heme ion can be inactivated by chelating agents such as EDTA and sodium tripolyphosphate salts (Eun et al., 1993). Ramanathan

and Dass (1992) studied the use of polyphenols like tannic acid, ellagic acid, α–tocopherol in ground fish and found that both tannic acid and ellagic acid showed comparable effects to that of BHT in raw and cooked ground fish. Also flavonoids extracted from natural sources such as quercetin, morin, myricetin and kaempferol have antioxidant effect on raw fish stored at 4°C (Ramanathan and Dass, 1992). Developing new methods to inhibit endogenous protease enzyme of fish muscle is a considerable issue that attracted the interest of food scientists. The inhibitory effects of certain compounds of fish endogenous enzymes in vivo have been exploited in the processing of fish with α_2-macroglobulin (Sareevoravitkul, 1995; Ashie et al., 1995). Simpson (1998) reported that combination of pressure with the broad spectrum protease inhibitor, α_2-macroglobulin, enhanced the capacity of endogenous enzymes to form stable fish gels.

ROO. (Peroxy radical) + AH_2 (Antioxidant) → ROOH + AH.	PRIMARY ATTACK
2AH. → AH_2 + A	DISMUTATION
ROO. + AH. → ROOH (lipid hydro peroxide) + A	SECONDARY ATTACK

Figure 2.3- Mechanism of action by antioxidants (Tuschiya, 1961)

2.5.7 High Pressure Technology and Its Principles

This is the technology of applying high hydrostatic pressure to materials by compressing the surrounding water and transmitting pressure throughout the product uniformly and rapidly (Hayashi, 1989). Investigation of effects of high hydrostatic pressure on food and microorganisms began in 1899 where it was used to increase the shelf life of milk (Hite, 1899). High pressure technology was commercialized in Japan in 1991 to sell high quality foods of low pH such as juices, jams, jellies, yogurts and salad dressing (Mermelstein, 1998).

Under pressure, bimolecules change according to the Le Chartelier-Braun's principle which implies high pressure promotes volume decreasing reactions and inhibits the volume increasing reactions (Hendrick et al., 1998). Due to this fact, at a relatively low temperature (0-40°C) covalent bonds are almost unaffected by HP where the tertiary and quaternary structures of molecules which are maintained chiefly by hydrophobic and ionic interactions are altered by high pressure $\geq$ 200 MPa (Hendrick et al., 1998). HP therefore, inactivates microorganisms, influences biopolymers and affects activities of enzymes while food quality factors such as nutrients or functional characteristics remain mostly unchanged. High pressure has offered new improvements to processing whereby its unique characteristics of keeping covalent bonds intact during compression, selective activation/inactivation of enzymes, bacterial destruction and its ability to prevent deterioration of sensorial attributes at a much higher rate than thermal processing, are just a few of the advantages that have made it a highly eligible candidate for producing high quality foods (Farkas, 1993). High pressure can be used in food processing in a similar way as temperature. For instance, hydrostatic pressure can induce gel formation in egg white and yolk, crude carp actomyosin, rabbit meat, and a suspension of soy protein by the application of 1,000 - 7,000 atm pressure at 25°C for 30 min (Farr, 1990). Numerous studies at McGill University (Montreal, Canada) have focused on high pressure processing and its effects on several food products. These include, HP application for fish gel-formation of superior quality (Sareevoravitkul, 1995), HP inactivation of enzymes affecting post-harvest texture deterioration (Ashie, 1996), HP inavtivation of pectin methylesterase in orange juice (Basak and Ramaswamy, 1996), HP processing of milk

and pork (Mussa, 1998), HP effects on milk rennet curd properties (Pandy, 2000), HP processing of trout and shrimp (Houjaij, 2000). Application of high pressure to prolong the storage life of chilled products appears to be a promising method for various kinds of foods, however all the experiments results have so far not been transferred to industry (Hurtado et al., 2000).

2.5.7.1 Effect of Hydrostatic Pressure on Protein and Enzymes

The primary and secondary structures of protein as formed by covalent bonds remain untouched by HP, while the tertiary structure of protein which is maintained mostly by hydrophobic and ionic bonds are affected by pressurization above 200 MPa, (Balny and Masson 1993). Also the quaternary structure of protein is affected by relatively lower pressure (150 MPa), because this structure is held together by weak non covalent bonds (Balny and Masson 1993). Conditions for denaturation vary for different proteins and reflect the structure of the individual protein. At high pressure, oligomeric proteins tend to dissociate into sub-units becoming vulnerable to proteolysis, while monomeric proteins do not show any changes in proteolysis with increase in pressure (Dickerson and Geis, 1969).

Enzymes are a special class of proteins with an active site, formed by the three-dimensional conformation of molecules (Hendrick et al., 1998). Biological activity of enzymes relates to their active site and any changes in the tertiary and quaternary structure of the active site are associated with volume changes and may therefore be affected by pressure (Heremans, 1992; Cheftel, 1992). Pressure also may inhibit the availability of energy by affecting energy-producing enzymatic reaction (Farr, 1990). High pressure can result in both activation and inactivation of enzymes depending on the pressure level and conditions. Usually at low pressure (100 MPa), enzyme activity may be enhanced, and at higher pressure the activity is inhibited (Curl and, Janson, 1950). Butz et al., (1994) and Gomes et al., (1996) explained that beside conformational changes, the de-compartmentalization caused by pressure results in enzyme activation.

When the food material is intact, there is compartmentalization between the enzyme and substrates. Application of low pressure in food tissue damages membrane and enzyme leakage leads to enzyme-substrate contact. Sometimes the activity of enzymes is enhanced or curtailed due to pH changes resulting from releasing of some components in the environment by the induced pressure.

High pressure influence on quality-effecting enzymes specially in fruits and vegetables, have been widely investigated (Ogawa et al., 1992; Butz et al., 1994; Eshtiaghi and Knorr, 1994; Knorr, 1995; Seyderhelm et al., 1996; Cano et al., 1997; Kim et al., 2001). Results from these studies suggest that pressure-induced changes in catalytic activity of enzymes differ depending on the type of enzyme, the nature of substrates, the temperature and length of processing. For example, mushroom and potato PPO seem to be very pressure resistant, high pressure levels at ~ 800-900 MPa are required for reducing the activity (Butz et al., 1994, Eshtiaghi and Knorr, 1994). While grape, strawberry, apricot and apple PPO have shown more sensibility to pressure. Pressure of about 100 MPa, 400 MPa and 600 MPa, were needed to inactivate PPO in apricot, strawberry and grape, respectively (Jolibert et al., 1994 and Amati et al., 1996). Pressure treatment up to 400 MPa at 25°C with 10 min holding time, increased the activity of polyphenoloxidase from peaches (Asaka and Hayashi, 1991), while pressurization/depressurization treatments at room temperature, caused a significant loss of strawberry PPO activity (60%) up to 250 MPa /15 min. The ability of high pressure to inactivate two important enzymes; lipoxygenase (enzyme produce off-flavor products) and polygalacturonase (enzyme critical in texture) in fruits and vegetables was studied by Shook et al., (2001). Pressure treatment at 800 MPa led to complete loss of activity of these enzymes, however PME appeared to be very resistant to pressure.

Basak and Ramaswamy (1996) studied the pressure destruction kinetics of PME in orange juice (pressure level, 300-400 MPa; pressure cycle, 1-3, and pressure-hold time, 30-120 min), and observed an instantaneous drop in activity of PME described as an instantaneous kill (IPK) when pressure pulse was applied, followed by a first order rate of inactivation during the pressure hold. This effect was more pronounced at higher pressure and lower pH. The initial drop in activity may be due to inactivation of heat

labile form of PME, while the remaining activity may be due to the heat stable form of PME (Goodner et al., 1998).

Proteolytic degradation is helpful in the tenderization of mammalian meats. High pressure technology can be used to reduce the ageing process. High pressure (1000 to 2000 atm) treatment of meat enhances the endogenous proteolytic activity that take place during meat conditioning by the release of proteases from lysosomes and by denaturation of the tissue protein (Ohmori et al., 1991). Homma et al., (1993), studied proteolytic enzymes activity (catheptic enzymes) in pressure treated meat crude extract. They reported that the activity of cathepsins B, D and L increased at 400 MPa, but tended to decrease at 500 MPa, while cathepsin H and aminopeptidase B activity decreased by increasing pressure. However for the preparation of some products (such as fish gels or pastes), proteolysis is not advantageous. Endogenous protease is one of the enzymes contributing to post-mortem texture changes or mushiness in muscle tissue of fish and crustaceans. Also degradation of myosin caused by proteolytic activity considerably reduces the gel-forming functionality of mince in surimi (Chung et al., 1994). Ashie (1996) studied the effect of high hydrostatic pressure on some texture-related enzymes, such as cathepsine C, trypsin-like, and chymotrypsin-like proteases, derived from both the bovine and the fish species. Ashie suggested seafood enzymes are more vulnerable to hydrostatic pressure inactivation than their bovine counterparts. Homma et al., (1994) also reported that bovine cathepsins B, D and L were stable to pressure up to 400 MPa. Homma et al., (1994) and Ohmori et al., (1991) reported an increase in proteolytic activity in beef muscle up to 300 MPa which they attributed to lysomal disruption. HP treatment has different effect on different proteases under similar conditions, e.g., HP at different pressure levels from 100-500 MPa with different holding times affects cathepsin D and reduces the activity comparing to non treated samples, while in the case of acid phosphatase, HP treatment increased the enzyme activity with all combination of pressure and time (Jung et al., 2000). Homma et al (1995) suggested that high pressure might enhance the activity of endogenous enzyme inhibitor as well, to result in inhibition after pressurization.

Studies on surimi-type products have shown that pressure induced gels are softer and more elastic than heat-induced gels. Furthermore, the pressure-induced gels retained the natural qualities (i.e., color and flavor) of the raw material without the formation of cooked color and flavor (Okamoto et al., 1990). High pressure eliminated the problem of 'modori' or gel weakening associated with the texture degradation of gels by heat-stable enzymes in surimi-type products. For example, Nagashima et al., (1993), demonstrated that high pressure treatment of squid mantle meat paste at more than 7,900 atm before two-step heating at 30°C for 30 min followed by 90°C for 30 min, could inactivate protease resulting in an increase of gel strength values. Chung (1994), reported that by combination of the two barriers, hydrostatic pressure and enzyme inhibitor, gel strength of Pacific whiting gels was significantly increased.

2.5.7.2 Effects of High Pressure on Microorganisms

Investigations of the effect of high pressure on foods and food microorganisms began in 1899 with studies on milk, meats and their microflora. It was shown that subjecting milk to pressure of 600 MPa for 10 min at room temperature reduced bacterial count from 10^7 cells/ml to 10^1-10^2 cell/ml. Meat subjected to pressure of 520 MPa for 1 h at 52°C was unaffected by microbial contamination after 3 weeks of storage (Farr, 1990). Microbial destruction has a higher rate of inactivation than enzyme inactivation (Smelt, 1998). Also it was found that gram-negative bacteria will be inactivated at lower pressure than gram-positive bacteria, with the pressure sensitivities of yeast intermediate to these two bacterial groups. The effect of high pressure on the viability of the microorganisms is, obviously, a combination of factors which cause changes in morphology, genetic make up, biochemical reactions, cell membranes and spore coats. High pressure alters the permeability of the cell membrane resulting in disturbed transport mechanisms leading to lack of nutrients and ultimately cell death (Houjaij, 2000). Changes induced to the cell morphology can be reversible at low pressure but irreversible at high pressure (Farr, 1990). Hayashi (1987), showed that the structure of the nucleus and cytoplasmic organelles in *Saccharomyces cerevisiae* were grossly deformed at pressure up to 31 MPa

and at 406 MPa nucleus could no longer be recognized. At pressure up to 310 MPa, large amounts of intercellular material leaked from the cell, while above 310 MPa loss of intercellular material was almost complete. In contrast to bacterial vegetative membranes, bacterial spores are very resistant to pressure. While pressure above 100 MPa causes rapid inactivation of many vegetative bacteria, bacterial spores may survive pressure above 1200 MPa. The structure and thickness of the bacterial spore coat are responsible for its high resistance (Lechowich, 1993). High pressure treatment and other mechanical stress may not directly inactivate dormant spores but induce their germination resulting in much lower resistance (Hayakawa et al., 1994). Hayakawa et al., (1994), demonstrated that microbial spores may be destroyed by a combination treatment involving elevated temperature (80°C) and high pressure (600 MPa). However this combination treatment seemed to be effective only when pressure was applied in short pulses. Hoover et al., (1989), reported that there is some concern regarding non-spore-forming pathogenic microorganisms which may show resistance to pressure destruction and pose risk of poisoning in food products. Among these non-spore-forming pathogenic microorganisms, *L. monocytogenes* Scott A and *Vibrio parahaemolyticus* are reported to exhibit resistance to pressure inactivation (Styles et al., 1991). Mussa et al., (1999), studied the HP destruction kinetics of *Listeria monocytogenes* Scott A in raw milk. They observed a double effect of pressure destruction of microorganisms consisted of (i) an instantaneous pressure kill (IPK) with the application of a pressure pulse with no holding time, and (ii) a subsequent first order rate of destruction during the holding time.

2.5.7.3 Advantages and Limitations of High Pressure

Some of advantages and limitations of using hydrostatic pressure method are:

(a) Some advantages of HP:

1. High pressure is not dependent of size and shape of the food.

2. High pressure is independent of time/mass, i.e., it acts instantaneously thus reducing the processing time.

3. It does not break covalent bonds; therefore, the development of flavors alien to the products is prevented, maintaining the natural flavor of the products.

4. It can be applied at room temperature thus reducing the amount of thermal energy needed for food products during conventional processing.

5. Since high pressure processing is isostatic (uniform throughout the food); the food is preserved evenly throughout without any particles escaping the treatment.

6. The process is environment friendly since it requires only electric energy and there are no waste products.

(b) Some limitation of HP:

1. Food enzymes and bacterial spores are very resistant to pressure and require very high pressure for their inactivation.

2. The residual enzyme activity and dissolved oxygen results in enzymatic and oxidative degradation of certain food components.

3. Most of the pressure-processed foods need low temperature storage and distribution to retain their sensory and nutritional qualities.

2.5.7.4 High Pressure with Combination of Other Methods

By taking advantage of high pressure technology in combination with other methods, the shelf life of seafood product can be extended for more days with better quality. For example, pressure treatments result in glossier and smoother surimi products with little shrinkage, and larger extensiblity (Simpson, 1998). Products formulated by high hydrostatic pressure technology also retain the nutrients, flavor, and freshness of the raw material better (Sareevoravitkul et al., 1996; Farr, 1990). Hurtado et al., (2000), reported that application of cyclic high pressure treatment to hake muscle at 200 MPa and in combination with vacuum packing, and chilled storage prolonged the shelf life by about 1 week, while pressurization at 400 MPa extended shelf life by about 2 weeks. High pressure in combination of vacuum packing also is used to extend the shelf life of prawns. Pressure treatment at 200 MPa and 400 MPa extended the shelf life of prawn from one week to 21 days and 35 days, respectively (Lopez-Caballero et al., 2000). Combination of high pressure with several chemicals used as melanosis inhibitors reduced black spots and microbial spoilage in prawn (Montero et al., 2001).

2.6 Rational and Objective of This Study

It is a common knowledge that seafood is generally much more perishable than other muscle protein foods. This is due to higher water content, the high free amino acid content, and the lower content of connective tissue of fish as compared to other flesh foods (Pedrosa-Menabrito et al., 1987).

Tuna fish is a well appreciated fish all over the world because of firm texture, flavorful flesh and high nutritional value (Ben-gigirey et al., 1999). Almost the entire tuna marketed in the world is canned, although the demand for fresh fillet and steaks is growing, probably because of interest in Japanese foods, such as sashimi, and popularity of tuna as a grilled item in restaurants (Ben-gigirey and Carven, 1998). In the United States, demands for premium quality and fresh tuna fish are increasing due to both an

increase in consumption and an increase import (Newman, 1998). However tuna fish possess unique physical and physiological attributes which render it susceptible to quality degradation. It has been implicated in scombroid or histamine poisoning because of high levels of free histidine in muscle tissue. Therefore preservation of quality and safety of tuna fish is an imperative task for seafood industry.

Due to limitations of current methods for producing fresh, additive-free and safe seafood products, investigation and application of novel physical treatments such as high pressure may offer great advantages over conventional methods. Numerous studies have been carried out on the quality and quality changes of canned tuna fish, while there are not many studies performed on the application of new preservation methods such as high pressure on the fresh tuna fish. Therefore the present study was aimed at investigating the effects of high pressure (220 MPa, 200 MPa, and 150 MPa) and holding time (15 and 30 minutes) on histamine formation and several physicochemical parameters that may limit the shelf life of refrigerated tuna. It should be noted that freshly caught tuna fish (yellowfin. *Thunnus albacares*) possesses a pink to deep red color and it is most desirable for tuna industry that the muscle should be pink or red with as little brown discoloration (Matthews, 1983). Hence, in the present study, pressure treatments levels were selected with the view to obtain raw appearance of tuna which can be commercialized as raw fish.

2.6.1 The overall objectives of this study were:

(1) To study the effects of high hydrostatic pressure on physical qualities such as texture and color of tuna fish

(2) To study the effects of high pressure on shelf life extension of fresh tuna during chilled storage (4±1°C) by evaluating the physicochemical/enzymatic changes

(3) To establish optimal high pressure treatment levels for maintaining freshness of tuna meat for commercialization

(4) To study the effects of high pressure on safety attributes of tuna related to histamine formation.

CHAPTER 3

MATERIAL AND METHODS

3.1 Sample preparation

Fresh yellowfin tuna (*Thunnus albacares*) were purchased from a local fish market (Peche Peche), Montreal, Quebec, Canada. Since different parts of fish flesh have different physico-chemical characteristics, only fish fillets from upper parts of fish were selected. Also, the dark parts of the flesh were completely removed in order to obtain uniform results. Samples were transported to the laboratory in crushed ice and stored in a cold room (4 ± 1°C) for 12 h, prior to packaging of samples. The whole fish fillet was skinned with a sterile knife, washed with distilled water, cut into small and uniform pieces weighing about 80 g for each. Samples were packed in dual-peel sterilization plastic pouches and vacuum-sealed immediately. After packing, samples (except the control samples) were subjected to high pressure processing at various pressure levels (150 MPa, 200 MPa, 220 MPa) and holding times (15 min, 30 min).

3.2 High pressure instrument

Prepared tuna samples were subjected to pressure treatment in a cold isostatic press (Model# CIP 42260, ABB, Autoclave System, Columbus, OH) consisting of a pressure chamber with an internal diameter of 10 cm and a height of 55 cm. The chamber contained a surrounding jacket which controls the temperature in the pressure chamber by circulating water at the desired temperature (Figure 3.1). The circulating water was obtained from a water tank where its temperature could be controlled manually by the operator (water temperature was adjusted quickly by adding ice). The medium used in the chamber as the hydrostatic fluid was a mixture of distilled water and 2% water soluble oil

(part no. 5019, ABB Autoclave Engineers, OH). Samples were placed inside the chamber immersed in the water-oil solution for the pressure treatments.

3.3 High pressure treatment

Samples were treated at 3 levels of pressure, 150 MPa, 200 MPpa and 220 MPa and 2 holding times, (15 min and 30 min). The temperature for the entire experiments was kept at less than 20°C by controlling the temperature of circulating water as well as media inside the chamber. After pressure treatment, the samples were immediately stored on ice in a cold room (4 ± 1°C). Fish samples including the control (un-pressurized samples) were randomly selected at time of analysis and were evaluated for color, texture, sensory attributes, pH, TBA values, total volatile nitrogen (TVB), drip loss, protease activity and histamine content. Samples were taken at different time intervals for different analyses during the storage period.

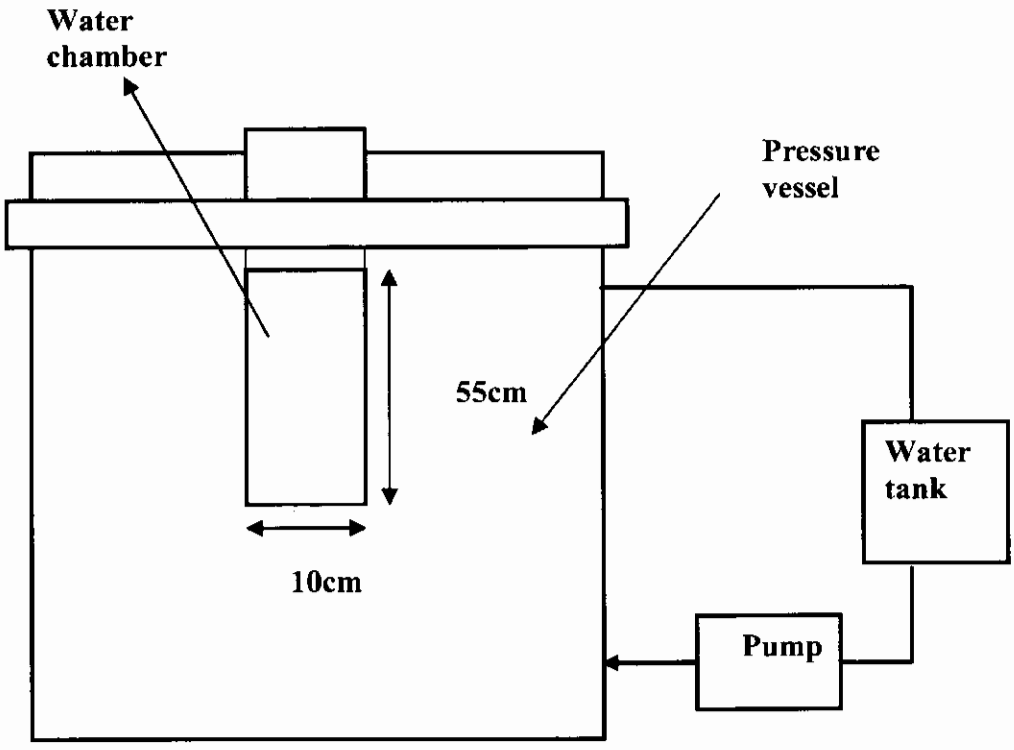

Figure 3.1 Schematic diagram of the high pressure unit

3.4 Physical attributes

3.4.1 Color measurement

A hand-held tristimuls color analyzer / Minolta Colorimeter (Chroma Meter II Reflectance Meter, Minolta Corporation, Ramsay, NJ) with illuminant D65, was used for color measurement. L*, a* and b* values of tuna samples (both pressurized and control samples) were determined. The L value represents lightness and is a measure of light intensity of samples; a* value or redness stands for the chromatic scale from green color (negative value) to red color (positive value); and b* value known as yellowness stands for chromatic scale from blue (negative b value) to yellow color (positive b value). (Figure 2.2 shows the color chart of L*, a* and b* values). The measuring head of the spectrocolorimeter had a 20 mm diameter measuring area and prior to every measurement calibration of instrument was performed against a Minolta white standard reflector. Also in order to compare the variations in L*, a* and b* values of samples at different stages of treatment and storage to those of the reference (fresh-untreated sample), a specific plate with a dark pink-orange color close to the color of fresh tuna sample was used as reference. In each measurement of L*, a* and b* values, 3 different readings from 3 different location on the surface of samples were taken and ultimately the mean value and standard deviation of L*, a* and b* and values were calculated.

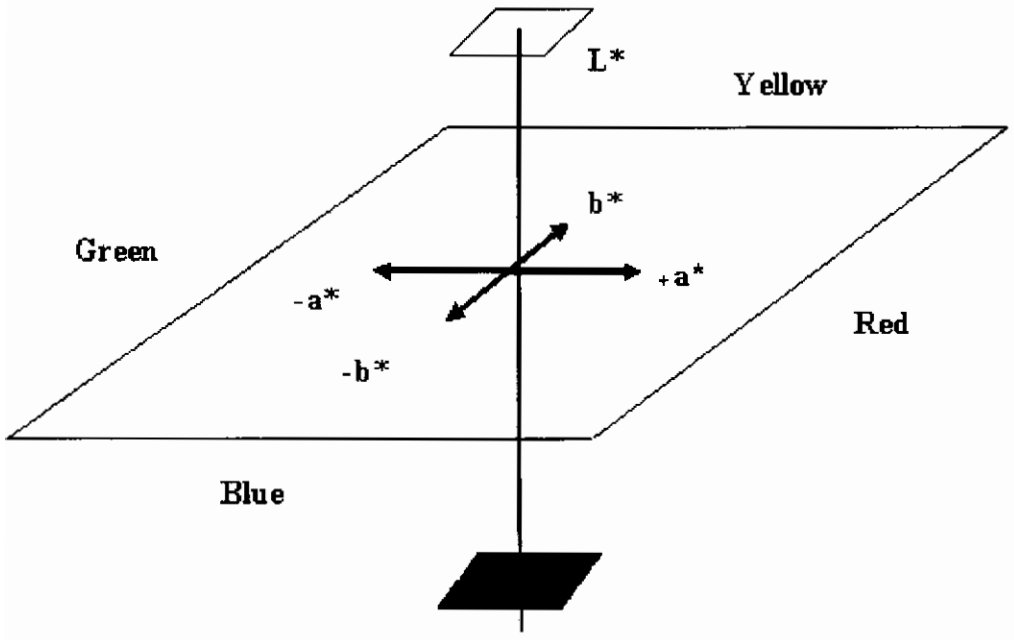

Figure 3.2 Color chart showing L*, a* and b* values

3.4.2 Texture attributes measurement (Firmness and Springiness)

Texture attributes of tuna samples such as firmness and springiness were measured using a Universal Texture Testing machine (Lloyd Model LRX, Fareham Hand, UK). Samples were cut into cubes of dimensions of 25 mm x 25 mm x 10 mm. A 50 N loaded cell with a speed of 25 mm / min was fitted to the instrument to carry out a compression test. The plunger was driven to compress the fish cubes by 35 percent of initial height of each sample. Both pressure treated and control samples were subjected to the compression test and a force deformation curve was obtained for each sample. The firmness and springiness of samples were obtained from the curve. Texture analysis was done in triplicates for samples and the average values were taken. Texture parameters obtained from the force deformation curve were based on following definitions:

Firmness (N/mm) = Slope

Hardness = Maximum Force (N) / Maximum Deformation (mm).

Springiness (%) = Recoverable Area (relaxation) / Total Area (compression)*100

The hardness measurement covered the whole deformation range of the typical force deformation curve while firmness was only measured from the linear portion of the deformation curve; therefore results are concentrated only on the firmness instead of hardness. A typical force deformation curve is shown in Figure 3.3.

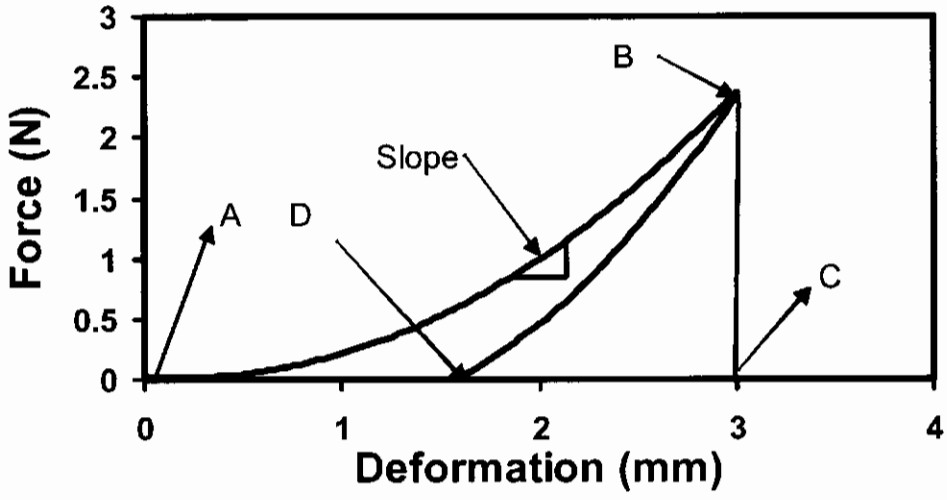

Fig 3.3 Typical force deformation cures

Where:

B= Maximum force

C=Maximum deformation

ABC=Total Area

BCD=Recoverable Area

3.4.3 Drip loss

About 1-3 g of fish samples were weighed and wrapped in Whatman filter papers No.4. Samples were placed in centrifuge bottles and centrifuged at 31000 g for 20 min at 4°C in a Beckmen refrigerated centrifuge (model J2-21, USA),. The difference in the weight of the samples after `and before centrifuging was an indicator of expressible moisture and was expressed as a percentage of the initial weigh of sample as follows.

Drip loss= $((w_2 - w_1) / w_1) . 100$

Where:

w_1 = **Initial weight of samples before centrifugation.**

w_2 = **final weight of samples after centrifugation.**

3.5 Chemical and enzymatic changes

3.5.1 pH measurement

About 10 ml of distilled water were added to 10 g of tuna samples and homogenized with a Polytron homogenizer for about 3 min at the speed of 8000 rpm. Samples were prepared in triplicate and pH was measured by inserting the pH meter electrode (Corning 220, pH meter) inside the fish homogenate and mean values were taken for pH result.

3.5.2 Total volatile base (nitrogen)

The method of Atonacopoulos (1992) was used for TVB-N determination of fish samples, with a slight alteration. About 20 g of fish tissue taken at regular intervals were homogenized with 40 ml of 7.5% of aqueous trichloroacetic acid solution for 1 min at high speed, using a Polytron homogeniser. The homogenate was centrifuged for 10 min at 1000 g and 4°C using a Beckman Refrigerated centrifuge (Model J2-21, United State). The homogenate was then filtered through a Buchner funnel using Whatman No. 3 filter paper. Steam distillation was performed by using 25 ml of the extract and 6 ml of 10% NaOH in a Kjeldahl-type distillation apparatus. Distillates were collected in 125-ml Erlenmeyer flasks containing 20 ml boric acid (4%) as trap solution until a total volume of 50 ml was obtained. Finally, the distillate was titrated with 0.02 N (v/v) hydrochloric acid to neutralize the solution from bluish color to pink color. Screen methyl red Bromo Cresol was used as indicator for the titration. The total volume of base-nitrogen in 100 g of sample was calculated based on the following formula. A control was run in the same way of distillation and titration. It contained everything in the mixture solution except the sample extract.

$$TVB\text{-}N = (14\ (V_x - V_y)\ N\ f\ /\ W)\ .\ 100$$

Where

V_x = **Titration volume of sample**

V_y = **Titration volume of control**

N = **Normality of acid**

f = **Dilution factor**

W = **Weight of sample**

3.5.3 TBA measurement

Thiobarbituric acid was determined by the method of Monez (2001). About 15 g of the fish samples were homogenized with 30 ml of TBA extraction solution (containing 7.5% trichloroacetic acid, 0.1% propylgallate and 0.1% EDTA), by homogenizing with a Polytron for about 4 min in an ice bath. Homogenates were centrifuged for 15 min at 1000 g and 4°C. The TBA reaction mixture was prepared by adding 5 ml of TBA reagent (thiobarbituric acid) to the same volume of prepared extract in a screw cap test tube and heated in boiling water for 1 h. After heating, samples were cooled under running tap water for 20 min and the spectrophotometric absorbance of TBA color reaction was measured at 530 nm against the blank in a UV-visible Spectrophotometer (U-200, Hitachi, Japan). The blank was made by mixing 1:1 of TBA reagent and water. TBA was calculated from standard curve obtained by reaction of 1,1,3,3 tetraethoxypropane with TBA reagent. Duplicate analysis was performed on all samples and TBA number was expressed in μg of malonaldehyde per g of fish.

3.5.4 Protease activity measurement

Protease activity was measured according to the method of Wang and Taylor, (1991) with some modifications. Crude enzyme was prepared by homogenizing 15 g of fresh tuna flesh with 100 ml of 0.2 M sodium phosphate buffer, pH 6.4, for 2-3 min with a Polytron homogenizer at medium speed. To prevent enzyme destruction from excessive heating during homogenization process, each sample was kept cool by placing the sample beakers inside an ice bath and stopping the homogenization every 20 second. The homogenate was centrifuged for 30 min at 12100 g at 40°C. The supernatant was used as the crude enzyme extract for the assay of protease activity. For the protease activity

reaction, 3.0 ml of 0.5% casein were used as substrate and added to 1.0 ml of the prepared crude enzyme. The reaction mixture was incubated for 30 min in a 45°C water bath. After the incubation, 3.0 ml of 5% (w/v) trichloroacetic acid (TCA) were added to the mixture to stop the reaction and precipitate proteins. The mixture was left to stand at room temperature for 1 h and then the precipitate was removed from the supernatant by filtration through Whatman No.1 filter paper. The absorbance (A) of the supernatant was measured at 280 nm in a 1-cm silica cell. Control sample was run by adding the crude enzyme after the TCA solution was added. Enzyme activity was expressed as A/ml per 30 min at 280 nm under assay conditions.

3.5.5 Histamine measurement

Histamine in tuna was determined by the AOAC (2000) official fluorometric method which comprised of four steps: (1) sample preparation and extraction, (2) sample purification, (3) O-Phthaldialdehyde (OPT) derivatization of purified samples to form fluorescence histamine derivatives, and (4) fluorescence measurement of histamine with a fluorescence spectrofluorometer (F-2000, Hitachi, Japan). In this method fluorescence intensity of derivatives was measured using fluorometer and histamine was quantified using external standards. Ten grams of sample were homogenated in 50 ml of 75 % (v/v) methanol for 2 min with a polytron homogeniser and extracted by heating in 60°C water bath for 15 min. The extract was cooled to 25°C and the volume was adjusted to 100 ml with extra methanol and filtered through Whatman No.1 filter paper. The methanol filtrate was collected and loaded onto an ion exchange poly-prep column (9 cm high, Concial 0.8 x 4 cm, polypropylene, BIO-RAD laboratories, CA, United States) with Dowex 1-X8, 50-100 mesh resin (Sigma Chemical Co., St. Louis, MO, United States). The flow rate of column was controlled at about 3 ml/min. For the purification process, 1

ml of extract was loaded to the column and eluted with distilled water in a 50 ml volumetric flask until about 35 ml were eluted. Then the column flow was stopped and the eluate was diluted with extra distilled water to the volume. O-Phthaldialdehyde (OPT) reaction mixture contained 10 ml 0.1 M HCl, 5 ml of eluate, 3 ml 1 M NaOH (that were added within 5 min), 1 ml 0.1 % (w/v) OPT solution and finally 3 ml 3.75 N H_3PO_4 which were added exactly after 4 min of adding OPT. The reaction mixture was mixed thoroughly after each addition and fluorescence intensity was recorded within 1.5 h. A blank was run with exactly the same solution of the OPT reaction mixture, except for the eluate which was replaced with 5 ml 0.1 M HCl. Fluorescence intensity of samples (I) was measured at excitation wavelength of 350 nm and emission wavelength of 444 nm. An external standard curve was prepared by using values from fluorescence intensity from different concentration of histamine solution (0.5, 1.0 and 1.5 μg / 5 ml aliquot). Standard regression was used to calculate the μg histamine / g fish which are expressed as mg histamine / 100 g fish in the results section.

3.6 Sensory evaluation

Samples of tuna fish muscle taken at regular intervals were placed in individual trays, and their sensory quality was evaluated by two untrained panelists in a bright and well-ventilated room. Sensory quality was assessed using scaling method of Soudan (1983). This method had a hedonic table which included 11 attributes related to fish. However in the present study on tuna fillet, only some of those characteristics (color, odor and general appearance) were used. Samples were scored, from 1 for the best quality to 6 for very poor quality. The mean value of the scores obtained for each aspect was used as sensory score of samples. Tuna was considered unsuitable for human

consumption and rejected by panelists when a final score of 3 or higher was obtained in assessment.

3.7 Statistic analysis

Statistical analyses of data were done using one way analysis of variance (ANOVA) and statistical analysis system (SAS/STAT, 1996) software.

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 Effects of high pressure on color

Unlike most fish species, tuna meat has a dark pink/red color and contains a considerable amount of myoglobin similar to red meats. Therefore it is very important that color of tuna fish be controlled if the shelf life is to be extended (Lopez-Galvez et al., 1995). Figure 4.1 is taken from tuna fillets samples- both control and pressure treated at different pressure levels and holding times (150 MPa, 200 MPa, 250 MPa for15 min or 30 min) immediately after high pressure treatment. It is obvious from the figure that all pressure treated fillets turned somewhat opaque and contrasted as the red/pink color in the non-pressurized samples (control). However samples subjected to pressure treatments of less than 200 MPa still maintained their dark red/pink appearance and resembled fresh tuna.

The L* values or lightness changes of pressure treated and control samples are also shown in the Figures 4.2a, 4.2b and 4.2c. The pressure treated tuna fillets generally lost their transparency and their color became lighter. L* values of all samples pressure treated above 150 MPa/15 min, increased progressively ($p<0.05$) with an increase in both pressure level and duration of pressure treatment. These results agree with those of other workers who reported that high pressure induces modifications in the appearance of fish muscle which generally became whiter when a pressure level of $\geq$100 MPa was applied (Oshima et al. 1993; Ashie and Simpson, 1996; Hurtado et al., 2000; Chevalier et al., 2001). This effect probably is attributed to the denaturation of the myofiblillar and sarcoplasmic proteins (Ledward, 1998). Color changes were also observed in the red muscle of tuna thawed by high pressure at 50, 100 and 150 MPa for 30 or 60 min (Murakami et al., 1992). Results also indicated that there were no significantl ($p>0.05$) increase in lightness values of samples treated at 220 MPa for 30 min and 15 min, MPa 200 for 30 min and 15 min and 150 MPa for 30 min during the storage from initial

values. While storage appeared to have a significant ($p<0.05$) effect on increasing the L* values or lightness of samples treated at 150 MPa/15 min versus control samples.

The a* value and b* value data are shown in Figures 4.3 and 4.4. The a* value data which is a red/green indicator, showed that the redness of all samples compared with control samples, declined significantly ($p<0.05$), with increasing pressure or holding time. The initial a* values of the pressurized samples also decreased with increasing storage time ($p<0.05$). These changes have been recognized to be specially due to conversion of the myoglobin in the fish tissue to met-myoglobin induced by both pressure application and oxidation during the chilled storage. Studies on pressurization of bovine meat in the range 200-500 MPa have shown that total extractable myoglobin decreased, while the proportion of metmyoglobin increased at the expense of oxymyoglobin (Chefel and Gulioli, 1997). Gibson and Carey, (1977) also noted that although the exact mechanism involved in color changes is not completely understood, pressure-induced denaturation of heme compounds like metmyoglobin, seems responsible in causing spectral shifts in such compounds. As the results for b* values indicate, yellowness of samples treated at pressures higher than 200 MPa for more than 15 min significantly ($p<0.05$) increased and changed from positive value in untreated samples to negative values for the treated samples. Also throughout the chilled storage up to 22 days, yellowness of all pressure treated samples as well as control samples significantly ($p<0.05$) decreased), however b* values of pressure treated samples were always higher than that of control samples.

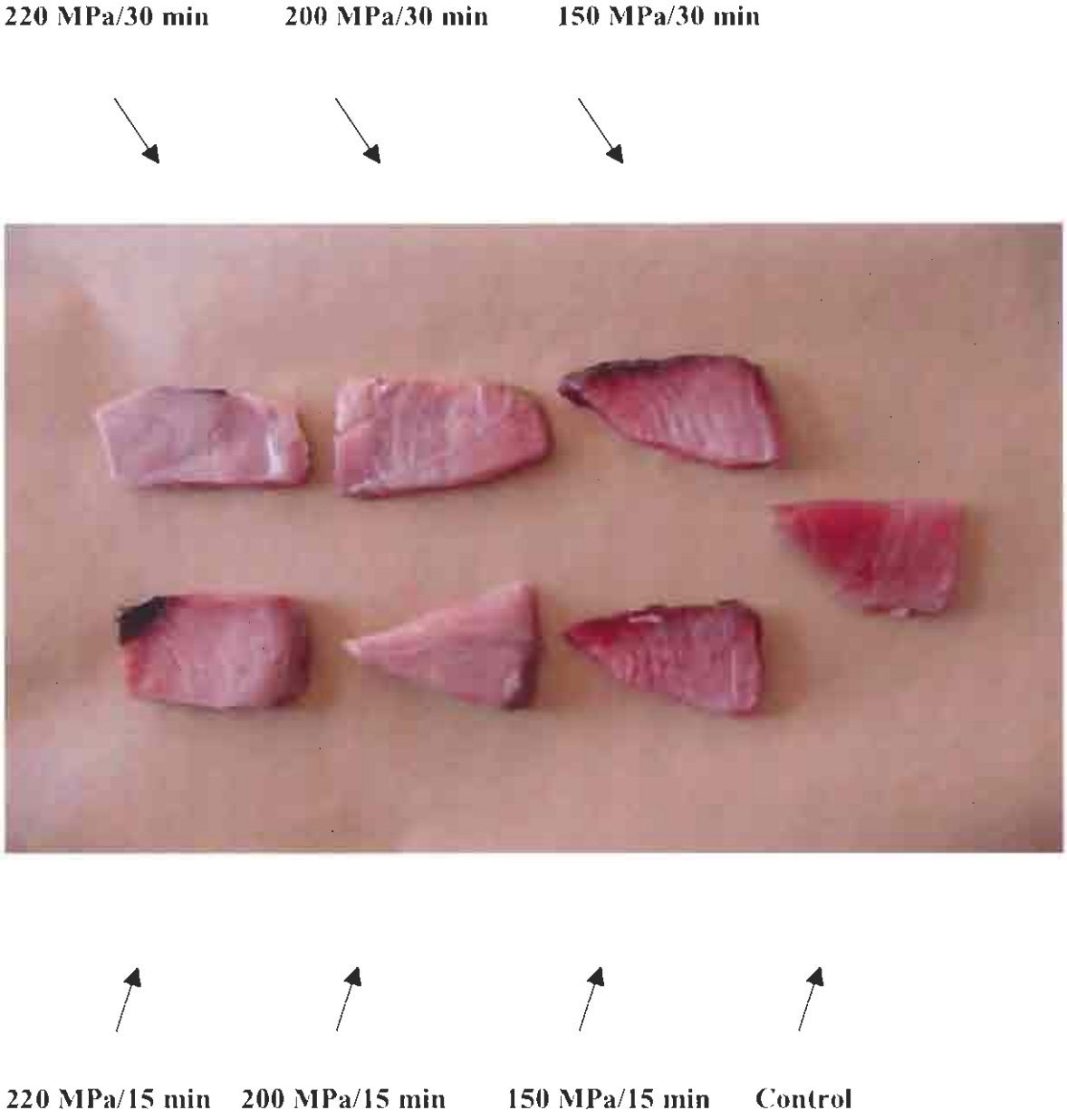

Figure 4.1- Pressure treated and control tuna fillets immediately after pressure treatment

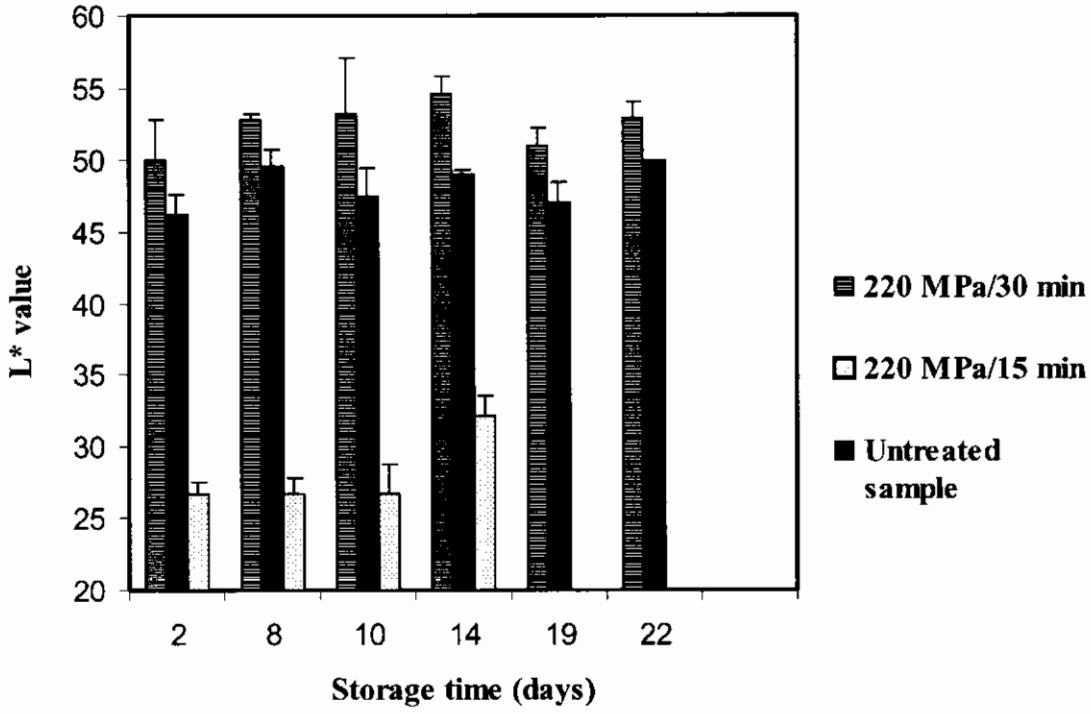

Figure 4.2a- Changes in L* value of samples pressure treated at 220 MPa/30 min and 15 min, compared with control (untreated samples) throughout the chilled storage.

Results are average values of 3 determinations

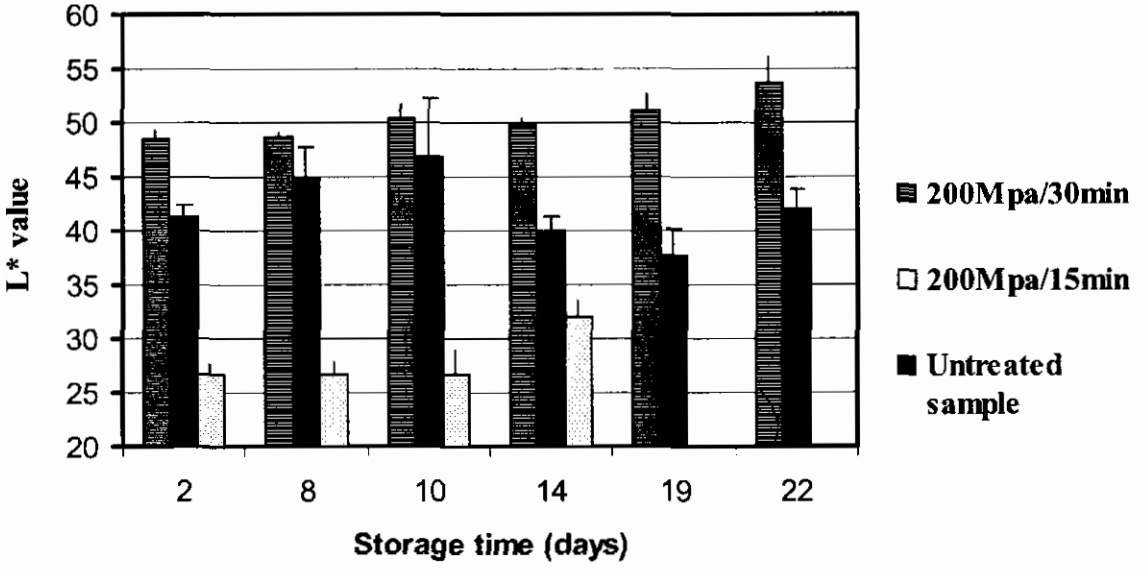

Figure 4.2b- Changes in L* value of samples pressure treated at 200 MPa/30 min and 15 min compared with control (untreated samples) throughout the chilled storage

Results are average values of 3 determinations.

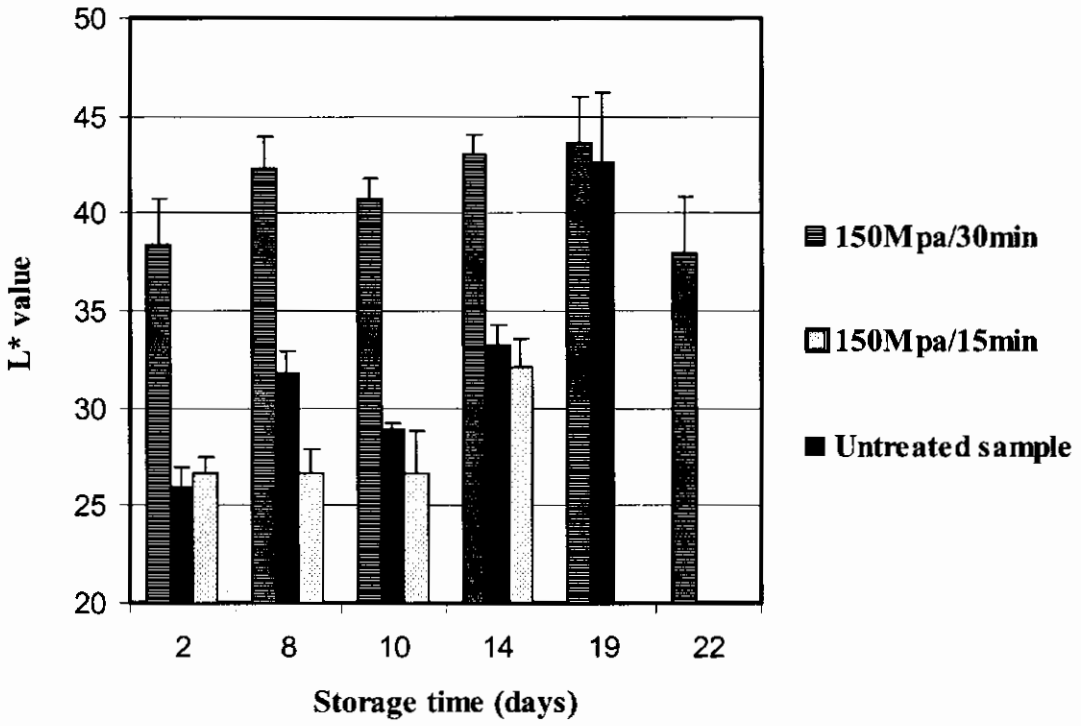

Figure 4.2c- Changes in L* value of samples pressure treated at 150 MPa/30 min and 15 min compared with control (untreated samples) throughout the chilled storage

Results are average values of 3 determinations.

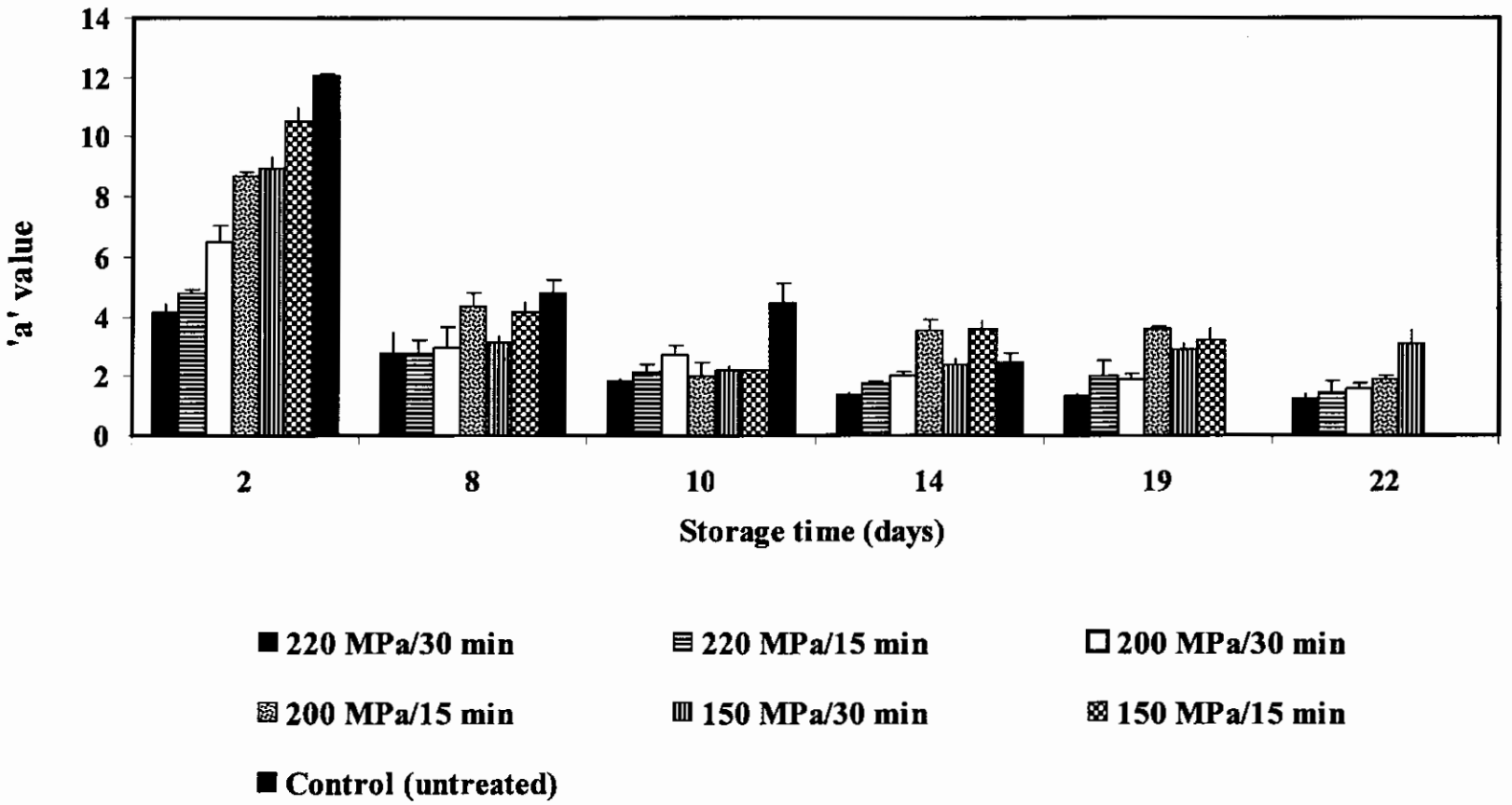

Fig 4.3- Changes in a* value of samples during chilled storage,

Results are from mean value of 3 determinations

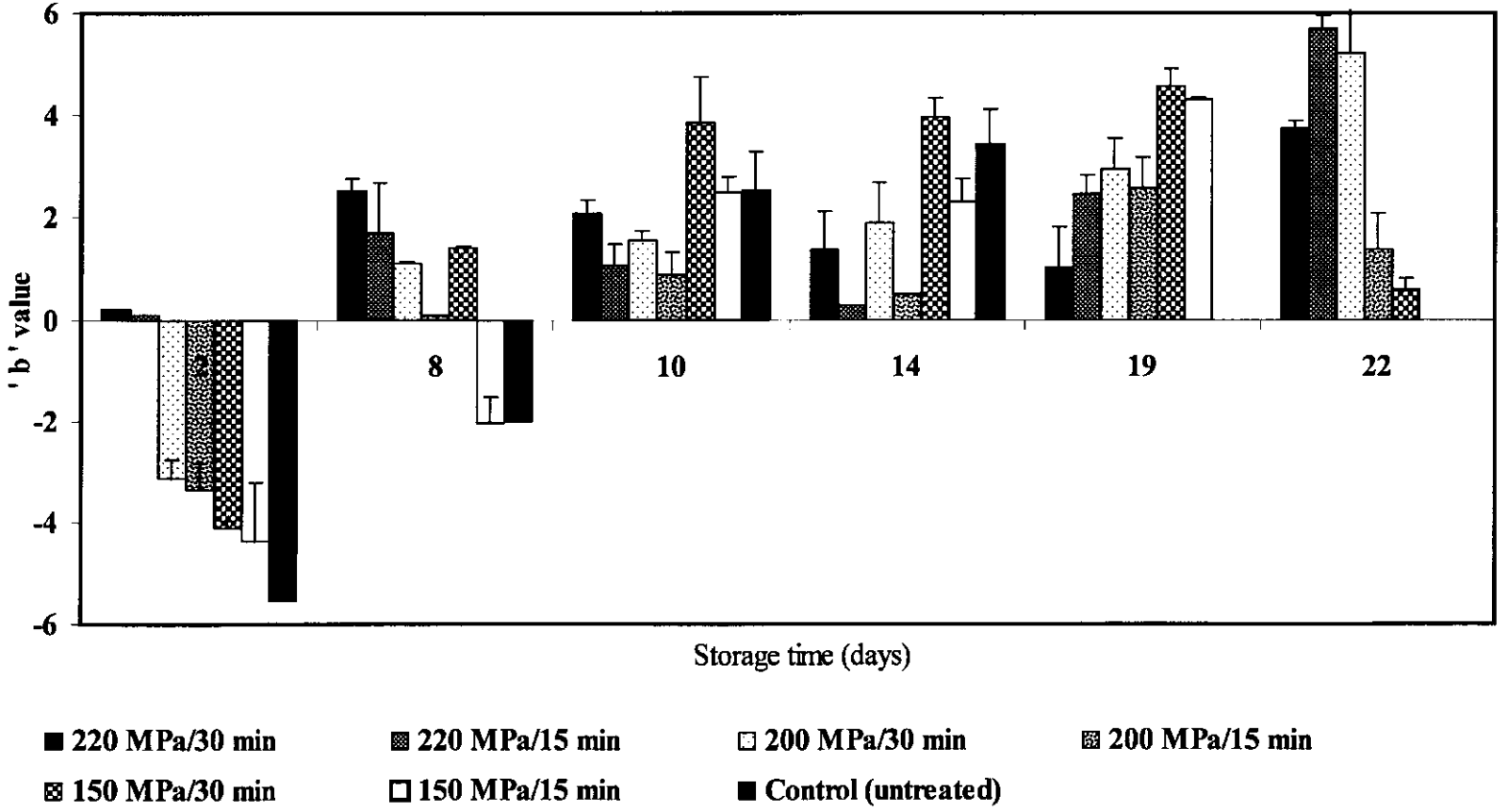

Fig 4.4- Changes in b* value of samples during chilled storage, Results are from mean value of 3 determinations

4.2 Texture analyses

Texture analyses (firmness and springiness) of the pressure treated and control samples were carried out to determine the effect of high pressure and storage on the texture attributes of tuna fillets. Typical firmness and springiness results of the samples are shown in Figures 4.5 and 4.6. The data indicate that pressurization significantly ($p<0.05$) increased firmness of all samples in all applied conditions, and higher pressure levels and holding times resulted in firmer muscle tissue. Typical results of springiness changes were also similar to the firmness changes. Pressure treatment above 150 MPa increased the springiness in fish tissue significantly ($p<0.05$).

These results are in agreement with the findings of Angsupanich and Ledward (1997) who studied texture profile changes of cod muscle treated at 100 MPa to 800 MPa. They reported that pressure treatment from 100 MPa to 400 MPa increased hardness and springiness of cod samples. They suggested these changes are probably due to unfolding of the actin and sarcoplasmic proteins and formation of new hydrogen bonded networks. Decrease in volume as a result of compaction enhances protein-protein interactions which lead to increase in bond formation causing an increase in tissue toughness and elasticity (Ohshima et al., 1993). Ashie, (1995), also found that pressure treatment up to 1000 atm for 10 min increased the firmness and elasticity in bluefish fillets and pressure treatment beyond this point led to a decline in toughness of muscles. He attributed the drop in tissue strength at higher pressure levels to the disruption of cohesive forces within the tissue by applying further compression force.

For the control samples, results showed that initial firmness and springiness generally declined throughout the storage and reached the lowest value by day 19 of storage. This decline in texture strength and elasticity suggests continued proteolytic enzyme activity in untreated fish tissue during the storage. After 14 days of storage, proteolysis in the control samples increased rapidly (see protease activity results). The firmness values of samples treated at 200 MPa/15 min and 150 MPa/30 and 15 min, also decreased slightly during storage time. The springiness of samples treated at 200 MPa/15

min and 150 MPa/15 min, also showed a decreasing trend. Changes in springiness values in samples treated at 150 MPa/30 min was not significant ($p>0.05$). Results also indicated that firmness and springiness of samples pressure treated at 200 MPa/30 min and 220 MPa/30 min and 15 min showed significant ($p<0.05$) increases after 10 days of storage. This increase in tissue hardness suggests protease inactivation in the samples pressurized at higher than 200 MPa/30 min. In addition, increase in firmness of samples can be due to loss of free moisture during the storage associated with decrease in water holding capacity of the samples induced by pressure. While for samples pressure treated at 150 or 200 MPa/15 min the decline in tissue firmness could be resulted from either incomplete inactivation or re-activation of protease enzyme activities during storage.

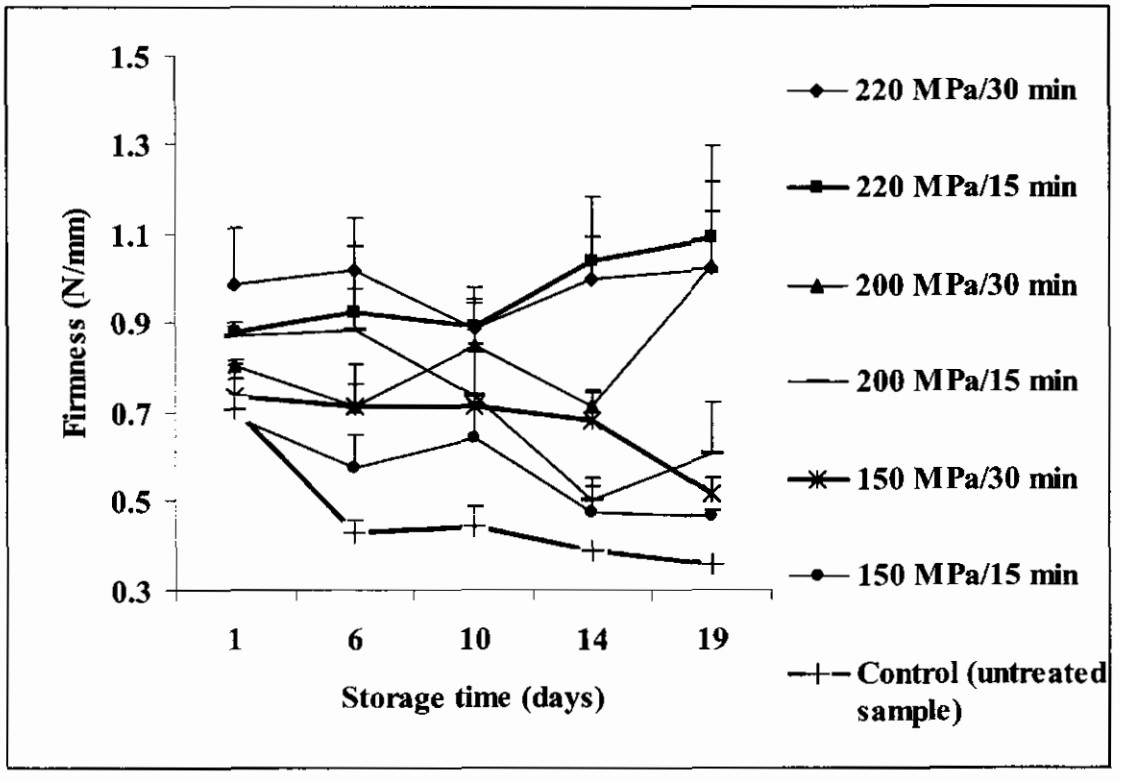

Figure 4.5-Changes in firmness of pressure treated and control samples throughout chilled storage. Results are average values of 3 determinations.

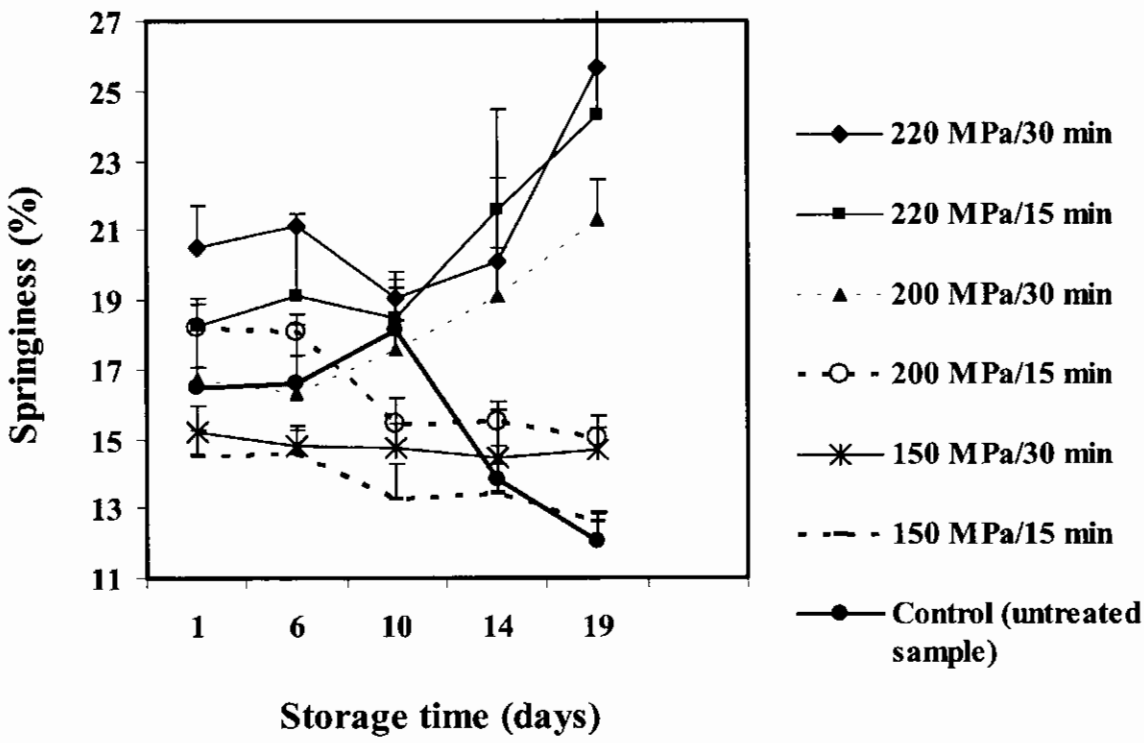

Figure 4.6-Changes in springiness of pressure treated and control samples throughout chilled storage, Results are average values of 3 determinations.

4.3 Drip loss

Drip loss is a factor adversely influencing juiciness, tenderness and flavor, and hence the consumers acceptability of muscle foods (Morrison, 1992). Figure 4.7 shows the changes in drip loss of tuna samples affected by different pressure treatment and chilled storage. Comparing the drip loss in control samples and pressure treated samples, it can be seen that high pressure treatment increased drip loss in all samples immediately after pressurization. Samples treated at 220 MPa/30 min and 15 min and 200 MPa/30 min resulted in the highest initial drip loss. Drip loss increased from 19% in control samples to 26% in samples treated at 220 MPa/15 min and to 23.3% in samples treated at 220 MPa/30 min. Several studies also reported that high pressure increases the drip loss in muscle tissue, as a result of compression to the myofiblillar networks and denaturation of myosin (Hastings et al., 1983; Ofstad et al., 1993; Elvelvoll et al., 1996). It is believed that high pressure promotes the drip loss and reduces water holding capacity, which is primarily related to disruption of electrostatic and hydrophobic interactions. These disruptions induce changes in protein-protein conformation leading to exudation from muscles which is collected as drip loss (Ikeuch et al., 1992; Gross and Jeenicke, 1994). Mussa (1998) also suggested that drip loss in pressure treated samples could be one of the reasons behind the increased in lightness of samples.

During chilled storage of the samples, drip loss increased in all samples. However it is obvious that the increase was significantly faster ($p<0.05$) in untreated samples (control) than that in pressure treated samples (increasing from 19% to 32%). Among the pressure treated samples, samples pressurized above 200 MPa/15 min showed a greater loss in drip, e.g., in samples treated at 220 MPa/30 min (from 26% to 35%), at 220 MPa/15 min (from 23% to 35%) and at 200 MPa/15 min (from 24% to 35%). This may suggest that generally pressure treatment of samples for less than 200 MPa/15 min may be able to control drip loss or increase the water holding capacity of samples in some extend, while pressure treatment at higher levels may cause a reverse effect on WHC. Macfarlane, (1985) stated that compression expels most of free fluid from muscle, which reduces the amount of expressed soluble proteins (ESP) and increases the water holding

capacity (WHC) of muscle. Effects of pressure on drip loss and cooking loss, like other physicochemical properties, differs depending on protein structure and nature of muscle tissue. Hurtado et al., (2000) reported that from pressure processing of hake muscle at 200 MPa and 400 MPa at 7°C, samples treated at 400 MPa showed a slower increase in drip loss during the chilled storage. There was no significant difference in drip loss evolution between the control and samples pressurized at 200 MPa.

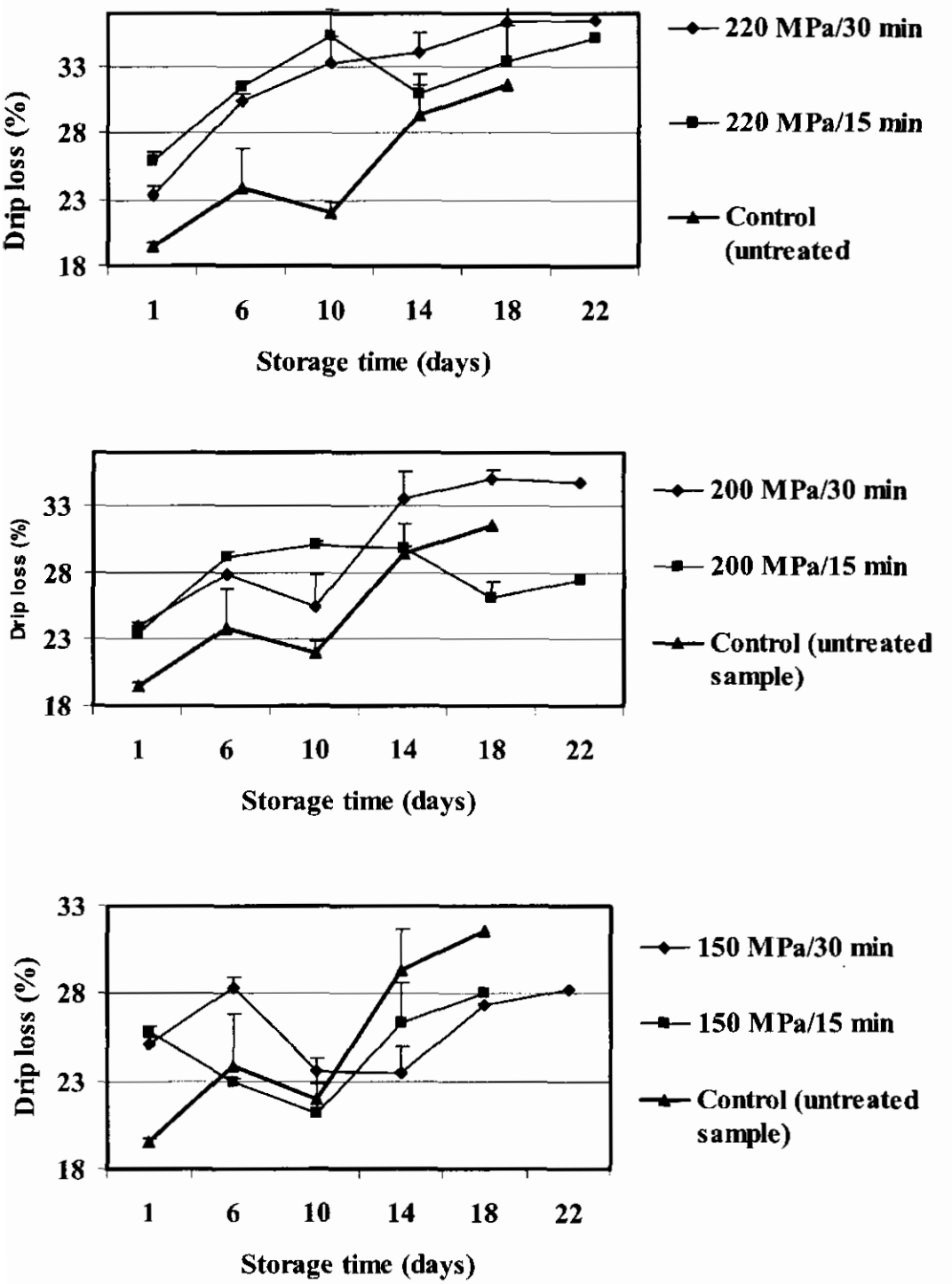

Figure 4.7- Drip loss changes of pressure treated and control samples during chilled storage. Results are average values of 3 determinations.

4.4 Proteolysis activity

Proteolytic activity (PA) of the pressure treated and control tuna samples during 22 days of refrigerated storage as judged by its activity against casein (casein 0.5%, pH=7 at 37°C) is illustrated in Figure 4.8. The absorbance (at 280 nm per 30 min) is directly proportional to the activity. Results have shown that changes in proteolytic activity of all pressurized samples and those of control samples immediately after pressure treatment is insignificant ($p>0.05$). Also proteolysis in pressure treated samples did not extensively increase during the entire chilled storage ($p>0.05$) and almost remained at a constant range. However, only in samples treated at 220 MPa/30 min an increase in PA was observed at day 22 of storage (this result is in contrast with the texture attributed to samples treated at 220 MPa/30 min). In the control or non-treated samples, there were slight changes in proteolytic activity from day 1 to day 14, and after 14 day of storage, proteolytic activity in the control samples increased progressively ($p<0.05$) and reached the highest value by 19 days of storage. The increase in protease enzyme activity of control samples after day 14 shows that proteolysis contributed to the degradation and tissue softening that led to decline in firmness observed from the texture studies with the control samples (Fig. 4.8).

Although proteolytic degradation is desired in the tenderization process of mammalian meat, it is not favorable in fish due to flesh softening. It is shown that enzymes implicated in seafood texture deterioration are more susceptible to inactivation by HP than their bovine counterparts (Ashie and Simpson, 1996). Bovine- chymotrypsin and cathepsin C were reported to be unaffected by pressure treatment up to 400 MPa (Homma et al., 1994), while high pressure treatment up to 300 MPa was able to reduce up to 88% of the activation of catepsin C and chymotrypsin-like enzymes from bluefish and sheephead (Ashie and Simpson, 1995). Present results suggested that high pressure processing of tuna fish at all applied pressure levels and holding times during the refrigerated storage were able to achieve some degree of control of proteolytic degradation in the intact tuna fish tissue, (an increase in PA of sample treated at 220 MPa/30 min, indicates the reactivation of protease after 19 days of storage in this

sample). In other studies various levels of high pressure were needed to control the proteolytic activity of fish tissue. For example, in a study of high pressure effects on cod muscle by Angsupanich and Ledward (1997), protease enzyme activities only decreased by increasing pressure level beyond 200 MPa, while in this study a noticeable decrease in activity was observed when samples were treated $\geq$ 150MPa/15min. Difference in critical high pressure conditions required for enzyme inactivation is explainable due to the fact that proteolytic activity varies among different fish species and even within the species (Wasson, 1992).

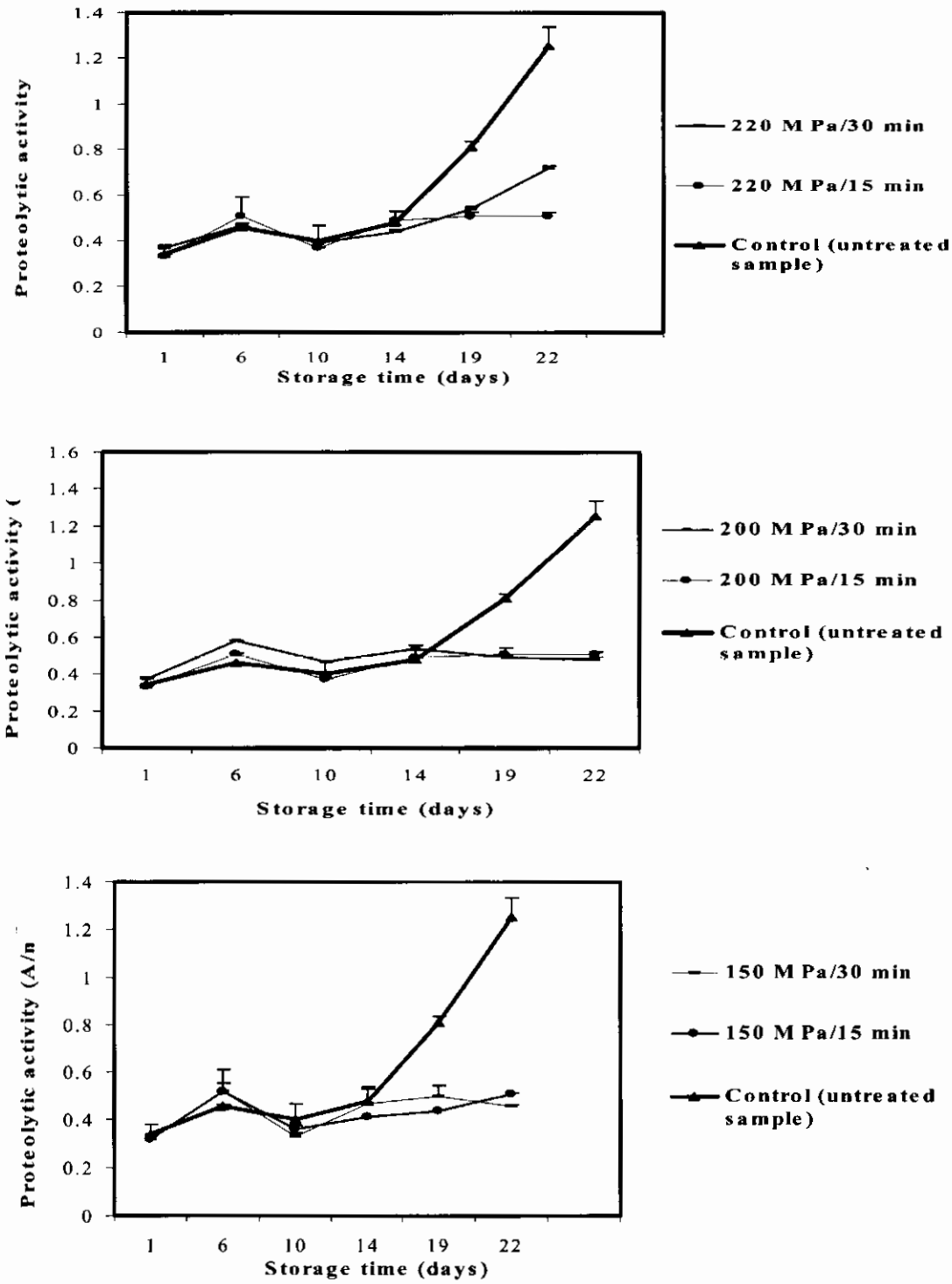

Figure 4.8- Proteolytic activity of pressure treated and control samples throughout chilled storage. Results are average values of 2 determinations.

4.5 pH

Fish freshness is traditionally judged by sensory methods, but several chemical indices such as pH and TVB, have also been proposed. Data presented in Table 4.1, illustrate the changes in pH values of tuna samples. According to CEUA (1998), samples with pH values within the range of 6.0-6.3, were considered normal and fresh for tuna fish. Pressure treatments indicated no significant ($p>0.05$) changes in initial pH values when pressure treated and control tuna samples were compared. Apart from control samples, pH values above 6.3 were attained in samples pressure treated at 150 MPa/30 and 15 min (No.5 and No.6), after 19 days of chilled storage and pH values of other pressure treated samples still remained in normal pH range. By day 22, pH values of all samples reached unacceptable level. In control samples, pH values changed more rapidly and exceeded 6.3, after 10 days of storage. These results are in agreement with the results from sensory evaluation and TVB values of tuna samples, as we can observe a relationship between the sensory decomposition and high values in pH and TVB results. Generally, in aerobically stored refrigerated fish, the increase in pH is due to TMAO reduction by spoilage bacteria and autolysis to TMA, which follows by conversion of TMA to other basic volatiles and further decomposition (Lopez-Galvez et al., 1995).

4.6 Total volatile bases (TVB)

TMAO reduction to TMA and other volatile nitrogenous compounds by spoilage bacteria in muscle during cold storage is an important factor in fish deterioration (Gokodla et al., 1998; Ryder et al., 1984). Therefore total volatile base-nitrogen (TVB-N) was measured and used as a fish freshness indicator. The TVB results for the pressure treated and control tuna fish samples are summarized in Table 4.2 and Figure 4.9. Differences in TVB values of pressure treated samples and those of the controls at day 1 of storage were not significant ($p>0.05$). After day 1 and throughout the chilled storage, the initial level of TVB (13-14 mg /100 g of muscle) significantly ($p<0.05$) increased for all the samples. However, the rate of TVB increase in the control (un-treated) sample was more pronounced than in the pressure-treated samples. A limit of 30 mg per 100g of muscle tissue has been considered as the upper limit above which seafood products are considered stale. Within this limit, fish is considered normal and fresh and above this limit up to 40 mg/100 fish is unsuitable for human consumption (Lang, 1979). Control samples reached the level 30 mg/100 rapidly by day 10, while TVB in other pressure treated samples remained under this level until day 14. By day 19 of storage, TVB levels were only higher than the unacceptable limit for samples pressure treated at 150 MPa/15 min and 30 min (No. 5 and No. 6), while the rest of samples showed an acceptable level of TVB.

The pressure treatment at 220 MPa/30 min and 15 min holding times had the highest impact in reducing the TVB values, while the HP treatment at 150 MPa/30 min and 15 min holding times had the least effect in reducing TVB values. Overall, from the TVB perspective, results indicated that high pressure treatment of tuna muscle could have a great impact in reducing TVB produced in muscle tissue resulting perhaps, from partial inhibition of bacterial growth involved in TMAO reduction. Consequently, shelf life extension of tuna fish from 10 to 19 days can be achieved when samples are subjected to high pressure processing at $\geq$ 200 MPa with holding time $\geq$15 min. However these results also suggest that none of applied pressure conditions did completely inhibit the TMAO reductase enzyme activity in tuna fish tissue.

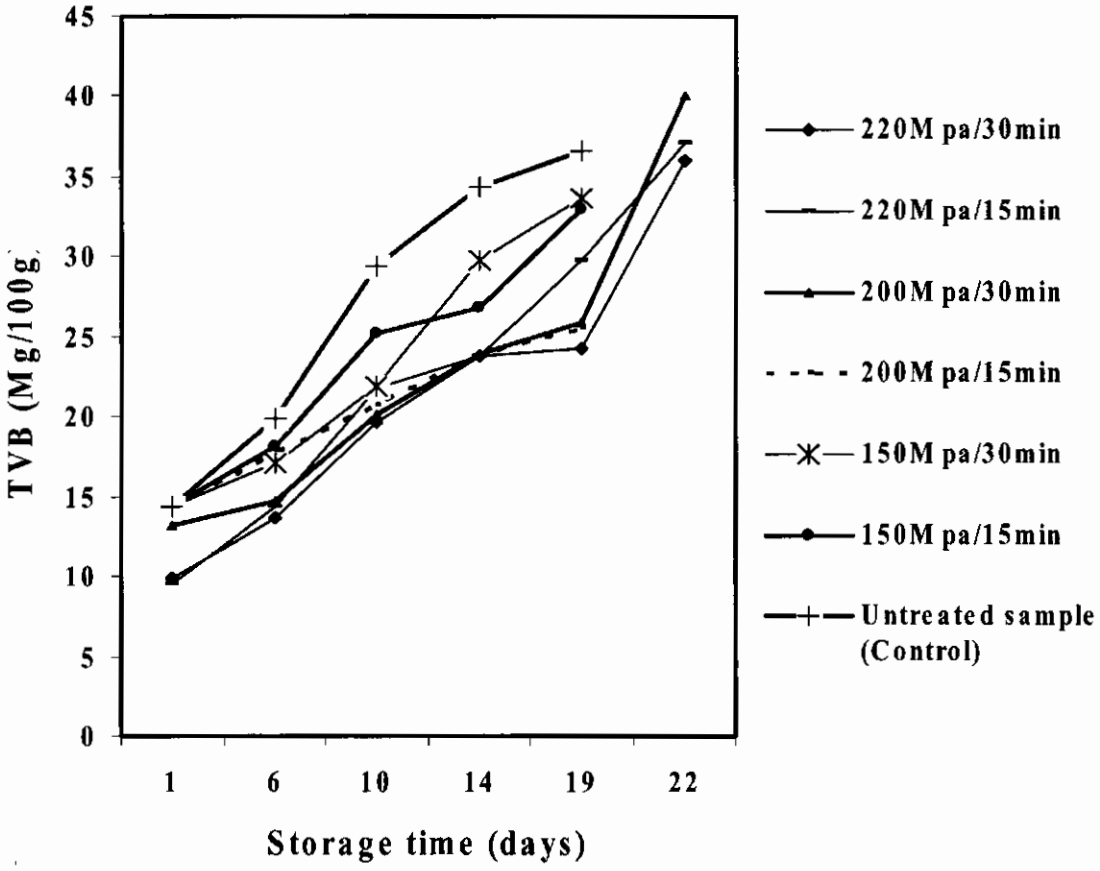

Figure 4.9- TVB values of tuna fish samples throughout chilled storage. Results are mean values of 3 determinations.

Table 4.3- TVB content (mg/100g) of tuna fish samples during refrigerated storage,
Results are from mean value of 3 determinations.
(nd = not determined)

Storage time (days) / **Pressure level/time**	1	6	10	14	19	22
220 MPa/30 min	9.93±0.387	13.7±0.129	19.64±1.034	23.78 ± 1.677	24.23± 0.592	**36.01±0.684**
220 MPa/15 min	9.5±1.010	14.43±0..529	21.8±.0.807	23.78±.0.762	29.75±.0.481	**37.11±2.24**
200 MPa/30 min	13.24±.0.775	14.73±.393	20.2±.0.775	23.89±1.293	25.87±1.010	**40.1±0.807**
200 MPa/15 min	14.34±0.448	17.71±0.233	20.76±0.976	23.89±.1.939	25.57±.0.807	nd
150 MPa/30 min	14.34±0.387	17.03±0.452	21.88±0.672	29.86±2.331	33.64±1.12	nd
150 MPa/15 min	14.34±0.387	18.1±0.129	25.24±0.224	26.86±1.933	33.04±0.56	nd
Control	14.35±0.592	19.93±0.129	29.44±0.807	**34.43±0.720**	nd	nd

4.8 TBA measurement

The TBA number indicates oxidative rancidity of fish. This parameter is measured spectrophotometically, by the reaction of malonaldehyde with thiobarbituric acid (TBA). Figure 4.10 shows changes in TBA values (in mg / Kg tuna, wet weight basis) at the beginning and throughout the storage period. Results suggest that high pressure treatment of samples did not affect the initial TBA values of the samples when compared with untreated samples ($p>0.05$). Also no consistent or regular pattern of changes in the TBA values was observed throughout the chilled storage of samples. However the TBA results showed that TBA levels of all samples were always lower than the 3 mg / kg (wet muscle weight) that is designated as acceptable levels in products. Therefore, it seems that this parameter is not clearly affected by the high pressure processing. This is crucial to note that high pressure effects on the oxidative stability of lipids in muscle food strongly depends on the presence of oxygen, other components of muscle, and temperature during and after pressure treatment (Cheftel and Culioli, 1997). Hence, the low TBA value of samples during the entire storage time is understandable due the fact that all our samples were kept in bags inside the cold room. Angsupanish and Ledward (1998) reported that pressure treatment below 400 MPa for 20 min at ambient temperature, had an insignificant effect on lipid oxidation in cod muscle. While studies by Cehvalier et al., (2000) have shown that trout muscle is more sensitive to pressure in terms of lipid oxidation. They noticed slight increase in TBA numbers of samples pressurized at 100 MPa for 15 min and 30 min and 140 MPa for 15 min when compared with control. Also by increasing the severity of pressure treatment, TBA levels increased, and at 200 MPa for a holding time of 30 min TBA numbers was 60% higher that that for a 15 min holding time. Several authors have suggested that acceleration effect of high pressure to lipid oxidation is due to denaturation of heme proteins by pressure which release metal ions promoting auto-oxidation of lipid in the pressure treated fish meat (Angsupanish and Ledward, 1998; Oshima et al., 1992).

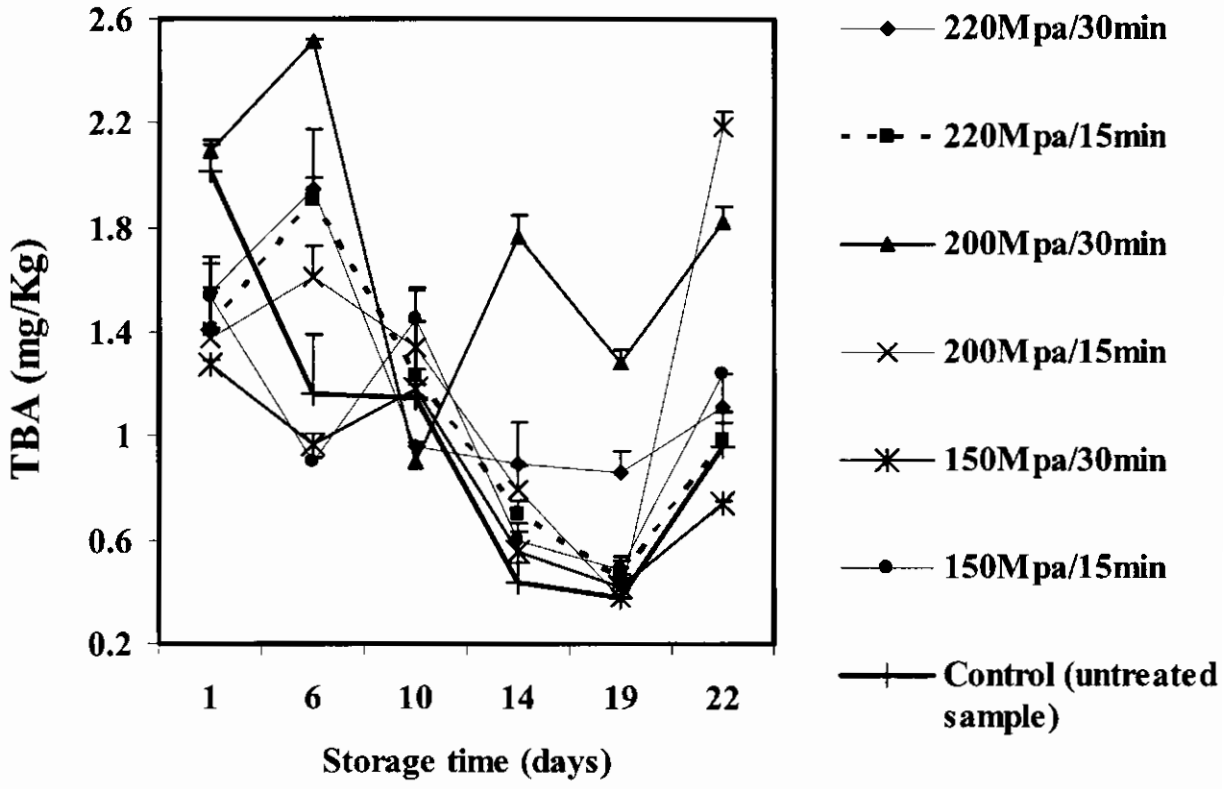

Figure 4.10- TBA measurements of tuna fish samples throughout chilled storage, Results are mean values of 3 determinations.

4.9 Histamine formation

Due to special effects of high pressure treatment on many biochemical substrates contributing to quality and safety of food material, it is necessary to evaluate the histamine content of tuna muscles after pressure treatment. The Food and Drug Administration (FDA) and the European Union established histamine levels of 50mg/100g and 100mg/100g, respectively, as hazard limit for tuna fish (Lopez-Sabater et al., 1995). Despite FDA high sanitized standard level, scombroid outbreaks are frequently reported all over the world.

Histamine content of pressure treated fresh tuna samples were measured immediately after pressure treatment and throughout chilled storage (Fig 4.11, Table 4.3). Interestingly, these results have shown that pressurization have a great impact on histamine formation of samples. This effect differs depending on the conditions of treatment. Pressure treatment did not induce any changes in histamine formation as histamine content was not detectable in either control and pressure treated samples on the first day. Also histamine content of samples did not noticeably change during 10 days of storage. After 10 days of storage, a dramatic increase in histamine content of the samples was observed; 220 MPa/15min, 200 MPa/30 min, 200 MPa/15 min, 150 MPa/30 min, 150MPa/15 min ($p<0.05$). Histamine content of the controls also increased significantly ($p<0.05$) after 10 days. Only one sample, treated at the highest pressure level (220 MPa) for a longer holding time (30 min), retained a low histamine level during the whole chilled storage. As for control samples, although histamine values increased significantly during storage time, it always remained less than FDA limit (50mg/100). These results suggested that pressure processing of tuna samples can have a great impact in histidine decarboxylase activity and histamine formation depending on pressure level and pressure treatment time as well as storage period. The present results showed pressure treatment of samples from 150 MPa/15 min to 220 MPa/15 min promoted histidine decarboxylase activity and enhanced histamine formation, while pressure treatment above these levels appeared to inhibit the histamine formation. High pressure effects on histamine and other biogenic amines production in seafood has not attracted a great deal of research attention.

Therefore for understanding the exact mechanism of HP effects in histamine formation, further investigation is needed. For example studies on the effects of high pressure processing on the crude and pure histidine decarboxylase enzyme activity, on histamine producing microorganism, on different ranges and conditions of high pressure processing, as well as conditions of fish storage can be recommended.

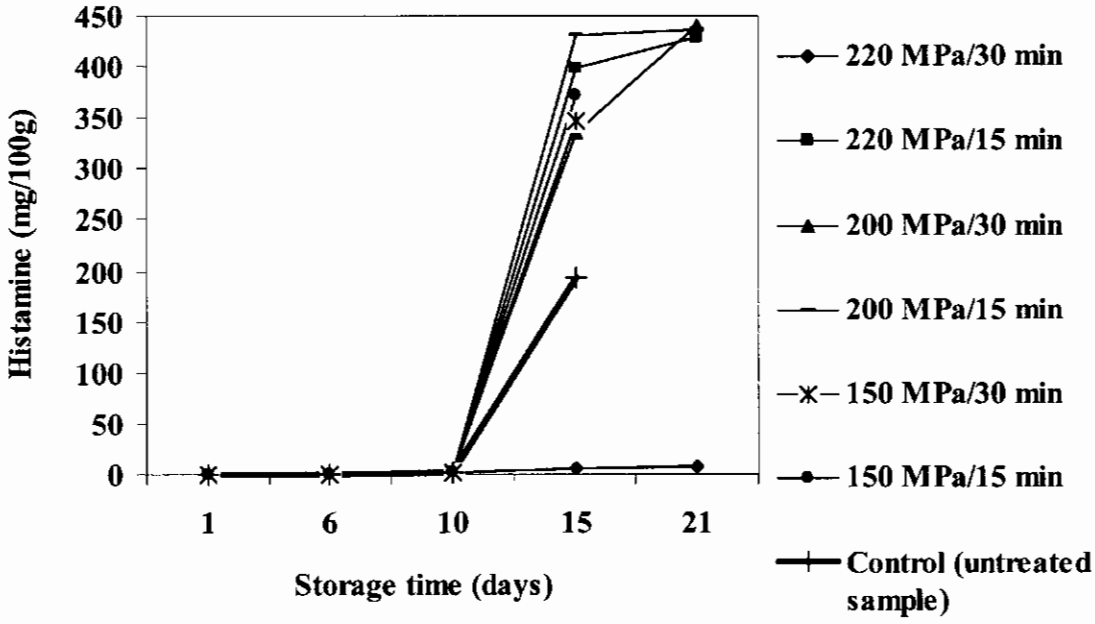

Figure 4.11- Changes in histamine content of samples throughout chilled storage, Results are mean values of 2 determinations.

Table 4.3- Histamine content of samples throughout chilled storage,

Results are from mean values of 2 determinations.

(nd = not determined)

Storage times(days) / Pressure level/time	1	6	10	15	21
220 MPa/30 min	0.51±0.005	1.2869±0.11	1.8411±0.013	**4.856 ± 0.416**	**8.388 ±0.831**
220 MPa/15 min	0.569±0.041	1.006±0.073	1.885±0.029	398.8±0.157	429.6±0.758
200 MPa/30 min	0.615±0.000	1.26±0.000	1.834±0.007	335.9±0.674	441.1±1.187
200 MPa/15 min	0.721±0.01	1.28±0.007	1.859±0.007	429.9±.674	435.7±.395
150 MPa/30 min	0.454±0.014	1.317±0.007	1.829±0.03	347.2±0.633	443.57±0.316
150 MPa/15 min	0.602±0.009	1.385±0.01	1.905±0.009	370.6±0.633	nd
Control (untreated)	0.452±0.001	1.266±0.02	1.836±0.013	192.7±0.365	nd

4.9 Sensory evaluation

Sensory evaluation was carried out by scoring method, where a score of 1 was for sample with the better quality, and 6 for very poor quality sample with all signs of decomposition. Mean values of scores obtained for each aspect (general appearance; color and texture and odor) were used as sensory score. Tuna is considered unsuitable for human consumption when a final score of 3 or higher is obtained in assessment. Sensory changes of samples are summarized in Table 4.4 and Figure 4.12. Results indicated that sensory quality of control samples declined at a much faster rate than pressure treated samples and reached the level of "poor" at day 6, while slight off-odor was evident and the muscle turned opaque with a gray/pink color. After 10 days of storage, the control samples were all showing signs of decomposition; offensive odor, grayish color with a soft texture, while the pressure treated samples were still considered relatively acceptable and better-preserved. Pressure treated samples from 200 MPa/30 min to 220 MPa/30 min remained acceptable even after day 15 of chilled storage. By day 21 of storage, all samples scored ≥ 3 and were unacceptable. These results show that sensory qualities of pressure treated samples were superior compared to the control samples. Further the shelf life of pressure treated samples treated at 220 MPa/30 min and 200 MPa/30 min was extended from 6 days to 18 days. Sensory analyses confirmed the results obtained from total volatile bases (TVB) and pH measurement of tuna muscle, but did not correlate with results from histamine content in tuna tissue.

Table 4.4- Sensory scores of samples after pressure treatment and throughout chilled storage Results are mean values of 3 determinations.

Storage time (days) / **Pressure levels/time**	**1**	**6**	**10**	**15**	**21**
220MPa/30min	1.4±0.14	1.4±0.17	1.5±0.23	2.5±0.70	**3±0.9191**
220MPa/15min	1±0.17	1±0.00	1.35±0.17	2.5±0.56	**3±0.98**
200MPa/30min	1.5±0.17	1.35±0.24	1.5±0.17	2.8±0.70	**3.5±0.35**
200MPa/15min	1±0.17	1±0.00	1.75±0.21	**3.5±0.56**	**4.5±0.21**
150MPa/30min	1.15±0.21	1.5±0.03	2.3±0.00	**4±0.14**	**5.5±0.35**
150MPa/15min	1±0.00	1.65±0.03	2.5±0.17	**4.5±0.35**	**5.8±0.0**
Control	0±0.00	2.5±0.31	**3.75±0.28**	**5.5±70**	**6±0.10**

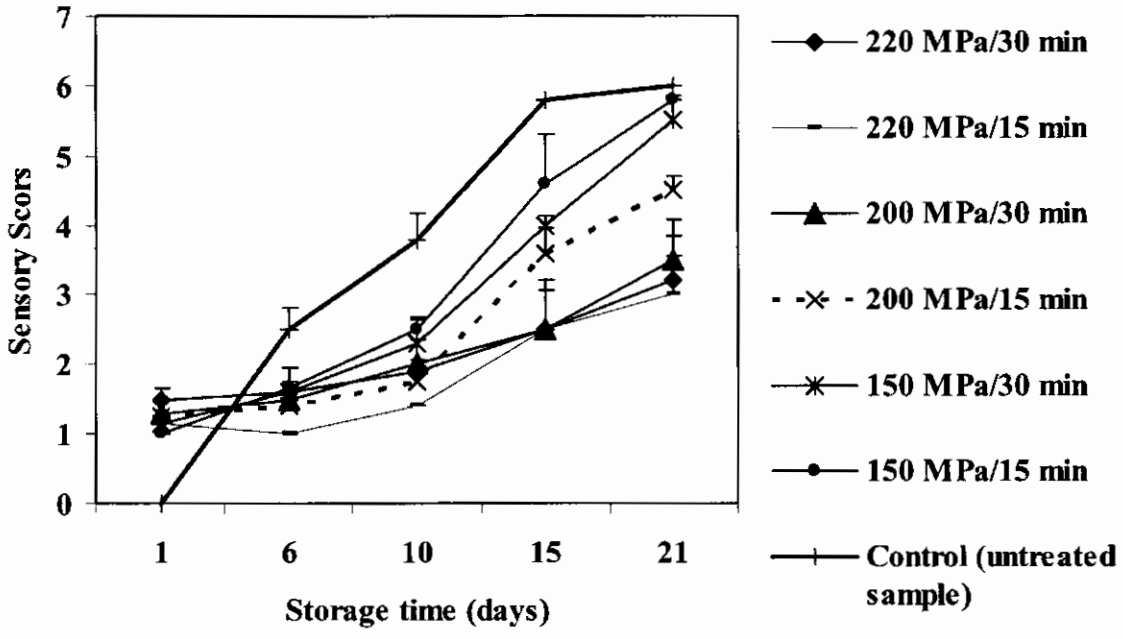

Figure 4.12- Sensory score of samples throughout chilled storage, Scores are average values of 3 determinations.

CHAPTER 5

CONCLUSION

1. Visual color analysis of tuna filets suggested that pressure treatment of samples under the conditions used in the study led to loss of glossiness and partial redness of samples. The L*, b* and a* values of samples correlated well with visual changes. In tuna processing when the purpose is to present the tuna fish as fresh and raw product, the applied pressure level should be less than 200 MPa/30 min. However higher pressure level may well be required for improving the quality and shelf life of fresh tuna. In this case use of color additives for enhancing the appearance or application of high pressure with combination of other preservation methods, can be recommended.

2. The texture of tuna fillet was greatly affected by HP processing. High pressure treatment above 150 MPa resulted in firmer muscles with higher elasticity. Firmness and springiness of untreated samples rapidly declined throughout the storage and reached the lowest value by day 19. During storage, slight decline in tissue firmness of samples pressure treated at 150 or 200 MPa/15 min was observed which was probably as result of either incomplete inactivation or re-activation of protease enzyme activity. While significant increase in firmness of the samples treated at 200 MPa/30 min and 220 MPa/30 min and 15 min, after 10 days of storage, can be related to protease inactivation and/or binding of moisture associated with decrease in water holding capacity. Texture firmness in pressure treated tuna fish is specially more desirable when longer storage time is required. Also HP effects on enhancing the texture of tuna fillets can be useful if freezing is to be applied, to reduce the texture degrading effects of freezing on fish muscle.

3. Drip loss results of control and pressure treated samples, suggested that high pressure treatment increased drip loss in all samples immediately after treatment. Specially, drip losses in samples treated at 220 MPa/30 min and 15 min and 200 MPa/30 min, were more obvious than others. However during chilled storage increase in drip loss was significantly faster in untreated samples (control) than in pressure treated samples. Our

results suggested that pressure treatment of samples below 200 MPa/15 min should be able to control drip loss or increase the water holding capacity of samples to some extent, while pressure treatment at higher this levels causes a reverse effect on WHC.

4. Proteolytic activities (PA) of the pressure treated samples remained roughly constant during the entire chilled storage. PA of control samples notably increased after 10 days of storage. Results indicated that HP treatments curtailed/reduced proteolysis in tuna muscles for almost 9 days (from10 days to 19 days). Therefore we can suggest that HP processing of tuna has potential to control endogenous protease activity in tuna muscle.

5. In the study of changes in pH value and total volatile nitrogen (TVB) content of tuna samples, we can see that results reinforce each other quite well. pH measurement showed that pressure treatment induced no major changes in initial pH values. Apart from the control samples, in which pH values rose more rapidly and reached above 6.3 after 10 days of storage, pH values above 6.3 were only attained in samples pressure treated at 150 MPa/30 and 15 min, after 19 days of chilled storage. HP treatment of tuna muscle showed great impact in reducing TVB produced in muscle tissue due to partial inhibition of bacterial growth involving in TMAO reduction. This impact was highest when samples treated at 220 MPa/30 min and 15 min and least in the samples treated at 150 MPa/30 min and 15 min. Shelf life of tuna fish increased by 9 days by subjecting samples to pressure processing at $\geq$ 200 MPa for $\geq$15 min, but none of applied pressure conditions could completely inhibit the TMAO reductase enzyme activity in tuna fish.

6. From a TBA point of view results suggested that application of high pressure up to 220 MPa/30 min, did not promote lipid oxidation in tuna flesh. Also during the entire 22 days of chilled storage (4 $\pm$ 1°C), TBA values of samples did not accelerate to unacceptable levels, which means that high pressure treatment at the applied conditions in fact was able to control the lipid oxidation in tuna fish muscles

7. HP *impact on histidine decarboxylase activity and histamine formation* differed depending on pressure level and pressure duration as well as storage period. After 10

days of storage, pressure treatment of samples at 150 MPa/15 min to 220 MPa/15 significantly promoted histamine production. Therefore these levels of HP treatments may pose a danger of histamine poisoning in tuna fish. Pressure treatment 220 MPa/30 min; have inhibited the histamine formation completely. Histamine values in untreated samples also increased significantly after 10 days of storage and rose above FDA limits (50mg/100g fish muscle) however, values were noticeably lower than those for samples treated below 220 MPa/30 min. This result is absolutely understandable since storage of samples under chilled condition (4 ± 1 °C), leads to inhibition of microorganism growth which are responsible for histamine formation in tuna fish.

8. Sensory qualities of pressure treated samples improved as compared to the untreated samples and shelf life of samples treated at 220 MPa/30 min and 200 MPa/30 min was extended from 6 days to 18 days. Sensory analysis of control samples reached the level "poor" on day 6, and by day 10 showed all signs of decomposition. While pressure treated samples from 200 MPa/30 min to 220 MPa/30 min remained acceptable even after 18 days of chilled storage. These results confirmed the results obtained from TVB and pH measurement of tuna muscle, while it did not correlate with results from histamine content in tuna muscles.

Overall conclusion

- Pressure level of 220 MPa and a 30 min holding time were optimal and most effective in prolonging the storage period of tuna muscle (up to 9 days), as well as in reducing the proteolysis activity, texture degradation, TVB and histamine formation.

- As several authors noted, there is no relationship between sensory attributes and histamine content and this probably is the reason behind of the frequently occurring incident of scombroid poisoning (Priebe, 1984; Smith, 1982; Taylor et al., 2002). Our results also support the above statement and therefore it can be concluded that sensory analysis of tuna samples is not adequate, and must not be used solely as an evidence of decomposition of tuna fish.

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