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The impacts of frozen storage and fatty acid oxidation on silver carp myofibrillar protein functionality

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DECLARATION

I ERASTO MLYUKA do solemnly declare that this research work entitled **"The impacts of frozen storage and fatty acid oxidation on silver carp myofibrillar protein functionality"** is of my personal research efforts under the supervision and guidance of Professor Chen Jie of the Protein Functionality research group in The State Key Laboratory of the School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu Province, P. R. China during the academic period 2007-2010, I further declare that this work has never been submitted to this University or any other institution of higher learning for the award of a degree and/or any other academic qualification.

Signed:... ..... Date: 2010.06.23

Erasto Mlyuka
(Msc.Candidate)

This is to certify that the research work presented herein entitled **"The impacts of frozen storage and fatty acid oxidation on silver carp myofibrillar protein functionality"** submitted by Mr. ERASTO MLYUKA for the award of a Master of Science degree in Agricultural Products Processing and Preservation Engineering is based on the results of studies carried out by him in the State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, P.R. China under my guidance and supervision during the academic period 2007-2010. I further certify that neither the research report nor any part thereof has been previously submitted for any degree and/or other academic qualification. This work is submitted for examination with my approval as University supervisor in Jiangnan University.

Signed: .....  .....Date: 2010-06-25

Prof.Chen Jie

(Supervisor)

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DEDICATION

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ABSTRACT

Silver carp (*Hypophthalmichthys molitrix)* is a freshwater species living in temperate conditions (6-28 °C) and its natural distribution is in Asia. It is generally cultured and marketed locally alive or fresh in most of the producing countries. China is the largest producer of silver carp in the world; however, its market price is relatively low compared to most other species, normally costs 8-10Yuan/kg. In order to keep the product longer and further extend the markets instead of just consuming it fresh, processing technologies for adding value and frozen storage are of significantly important for this fish species.

In this thesis, the report is mainly focused on the study done on isolation of myofibrillar protein from silver carp; exposing the isolated myofibrillar protein to oxidative environment simulated using an iron oxidation model system to mimic the oxidative stress during processing and frozen storage. Also the impacts of the iron oxidation model system, fatty acid oxidation and frozen storage on protein functionality of silver carp myofibrillar protein isolate were evaluated.

Oxidative damage to silver carp myofibrillar proteins isolate (MPI) was investigated by measuring changes in physico-chemical, and functional properties after exposure to iron-catalyzed oxidation system (IOS). Iron oxidized MPI exhibited an increase in carbonyl content and dityrosine, which were significant and negatively correlated with protein solubility (PS) (r=0.85), (r=0.80) and gel strength (GS) (r=0.95), (0.93), respectively, however the decrease in total thiol group content was significantly and positively correlated with PS (r=0.77) and GS (r=0.89). These led to significant changes *($P<0.05$)* observed in the protein and functional properties of oxidized MPI. Sodium dodecyl sulfatepolyacrylamide gel electrophoresis demonstrated that IOS resulted in a major loss of myosin and actin associated with formation of protein polymers as supported by gel-permeation chromatography (GPC) results. The total fat content of silver carp MPI was 0.6%, it is mainly composed of unsaturated fatty acids of which about 34% constituted by eicosapentaenoic acid, (20:5ω-3), docosahexaenoic acid, (22:6ω-3), and linolenic, (18: 3ω-3) as the main ω-3 poly-unsaturated fatty acids (PUFAs) as well as linoleic acid, (18:2ω-6) and arachidonic acid, (20:4ω-6), the main ω-6 PUFAs in fish , unfortunately PUFAs are prone to oxidation producing reactive oxygen species capable of modifying protein structure. This study suggests that the decreased functionality of proteins in muscle foods exposed to an oxidative environment could be due to chemical and physical changes resulting from oxidation reactions.

It was found that silver carp myofibrillar protein is susceptible to an iron-catalyzed oxidation causing a significant loss of its functionality, thus a study was carried out to

determine the susceptibility of silver carp protein to natural oxidants (fatty acids or fatty acids oxidation products) in frozen storage with respect to its functionality. Frozen stored myofibrillar protein isolate with 0.6M NaCl or myofibrillar protein isolated from previous frozen stored whole fish and fish mince of silver carp at -18°C for 90 days were assessed for protein and lipid oxidation with regard to protein functionality. The addition of sodium chloride to 0.6M at pH6.5 improved protein functionality especially water holding capacity of frozen stored myofibrillar protein isolate from 5.3 to 6.4mL/g MPI after 90 days of frozen storage. However, the differential scanning calorimeter results showed that, sodium chloride significantly increased thermal susceptibility of myosin from 48.12 to 46.40°C with 0.1M NaCl and 0.6M NaCl frozen stored for 90 days, respectively. Whole fish frozen storage was more susceptible to oxidation compared to fish mince and myofibrillar protein isolate due to their different lipid content. On contrary, myofibrillar isolated from frozen whole fish showed significant changes in protein functionality may be due to great loss of amino acid such as cysteine, lysine, histidine and methionine during frozen storage.

On the other hand investigation was done on stabilization and oxidation protection of silver carp MPI stored at –18 °C, 90 days and composed of MPI, 8% cryoprotectants (4% sucrose and 4% sorbitol) with or without antioxidants (0.2% ascorbate, 0.2% α-tocopherol, or their combination) and packed in a polyethylene bag, sealed under air. MPI without cryoprotectants and antioxidants was the control. Compared with the control, cryoprotectants increased PS (protein solubility), WHC (water holding capacity), OHC (oil holding capacity), EC (emulsification capacity) and GS (gel strength), also, the cryoprotectants and/or antioxidants decreased MPI oxidation susceptibility as well as susceptibility of myosin to thermal denaturation. After 30 days of frozen storage, there were no significant differences *($P>0.05$)* of storage time on alteration of protein functionality and actin susceptibility to thermal denaturation between cryoprotectants and the control. Antioxidants minimized oxidation effects on MPI frozen storage.

Therefore, the present study reveals that, improvement in the functional properties of silver carp MPI were greatly influenced by cryoprotectants and antioxidants, this reflect a great role played by sucrose/ sorbitol and antioxidants in protecting native protein structure to a greater extent owing to impact of frozen storage and oxidation respectively.

Keywords: silver carp, oxidation, protein functionality, cryoprotectants, antioxidant, frozen storage.

摘要

鲢鱼（*Hypophthalmichthys molitrix*）是一种生活在 6-28℃温度条件下的淡水物种，自然分布于亚洲。在大部分生产国，鲢鱼经养殖后通常在当地范围内以新鲜活鱼的方式进行市售。中国是世界上最大的鲢鱼生产国，然而，其市场价格相对低于大多数其他鱼种，通常仅售 8-10 元/kg。为使产品保存更长久，并进一步扩大市场而非仅新鲜消费，增加价值及延长冷冻贮藏的加工工艺对此鱼种尤为重要。

本文着重研究了从鲢鱼中分离肌原纤维蛋白，并将其暴露在铁氧化模型系统模拟的氧化环境中，以模拟加工和冷冻贮藏过程中的氧化应激。此外，还评估了铁氧化模型系统和冷冻储藏对鲢鱼肌原纤维蛋白的功能性所产生的影响。

通过测定暴露于铁催化氧化系统（IOS）中鲢鱼肌原纤维蛋白提取物（MPI）的物理-化学以及功能性质的变化来研究 MPI 的氧化损伤。对于铁氧化的 MPI，其羰基和二酪氨酸含量均增加，这与蛋白溶解性（PS）（r=0.85）、（r=0.80）和凝胶强度（GS）（r=0.95）、（r=0.93）呈显著负相关。但总巯基含量的降低与 PS（r=0.77）和 GS（r=0.89）呈显著正相关。这些均导致氧化的 MPI 及其功能性质发生显著变化（P<0.05）。聚丙烯凝胶电泳表明，IOS 导致肌球蛋白和肌动蛋白损失严重，并形成蛋白聚合物，这从凝胶渗透色谱(GPC)的结果可以得到证实。鲢鱼 MPI 的总脂肪含量为 0.6%，主要由不饱和脂肪酸组成，其中约 34%为 EPA (20:5ω-3)、DHA (22:6ω-3) 和 ALA (18: 3ω-3)（它们是鱼中主要的 ω-3 PUFAs）以及 LA (18:2ω-6) 和 AA (20:4ω-6)（它们是鱼中主要的 ω-6 PUFAs）。不幸的是，PUFAs 易于氧化产生能修饰蛋白结构的活性氧。此研究表明，暴露于氧化环境的肉制品中蛋白质的功能性质降低，可归因于氧化反应导致的物理化学变化。

研究发现，鲢鱼肌原纤维蛋白易于受到铁离子催化的氧化反应攻击，并导致其功能性质大量损失。因此，本试验测定了鲢鱼蛋白在冷藏储存过程中对天然氧化剂的敏感性，以及对应其功能性质的变化。分别测定了在-18℃ 储存了 90 天的加入 0.6M NaCl 的鲢鱼肌原纤维分离蛋白以及从冷冻的全鱼或鱼糜中分离得到的肌原纤维蛋白的蛋白氧化情况和脂类氧化情况，及对应的蛋白功能性质的变化。在 pH6.5 下，加入 0.6M NaCl 后，蛋白功能性有所提高，尤其是冷冻肌原纤维分离蛋白的持水性，冷藏 90 天后，其持水性从 5.3 提高到 6.4mL/0.5g 蛋白。然而，差示扫描量热结果显示，NaCl 能够明显的提高肌球蛋白的热敏感性，贮存 90 天后，添加 0.1 MNaCl 和 0.6M NaCl 的蛋白的热变性温度从 48.12 ℃ 降至 46.40℃。由于具有不同的脂肪含量，冷冻的全鱼比鱼糜和肌原纤维分离蛋白更易被氧化。相反的，从冷冻全鱼中分离的肌原纤维蛋白的功能性质变化显著，原因可能是由于储藏过程中大量氨基酸的流失所造成，例如半胱氨酸、赖氨酸、组氨酸和甲硫氨酸等。

因此，论文进一步研究了鲢鱼 MPI 在冷冻贮藏过程中的稳定性和氧化保护作用。将 MPI 与 8%冷冻保护剂（4%蔗糖和 4%山梨醇）以及抗氧化剂（0.2%抗坏血酸、0.2%α-生育酚或

其组合）按照不同比例混合，密封包装于聚乙烯袋中，于-18℃储存 90 天。以不含冷冻保护剂和抗氧化剂的 MPI 作为对照。与对照组相比，冷冻保护剂增加了蛋白溶解性、持水能力、持油能力、乳化性和凝胶强度；另一方面，冷冻保护剂、抗氧化剂以及两者合用都降低了 MPI 的氧化敏感性，以及肌球蛋白的热变性敏感性。储藏超过 30 天后，对于添加冷冻保护剂组和对照组来说，贮藏时间对蛋白功能性质的改变及肌动蛋白的热变性敏感性没有显著差异（$P>0.05$）。抗氧化剂将氧化对 MPI 冷冻储存的影响降到了最低。

综上，本研究表明添加冷冻保护剂和抗氧化剂能够明显提高鲢鱼蛋白在冷冻贮藏中功能性质，蔗糖/山梨醇和抗氧化剂通过对冷藏过程和氧化过程的影响，保护了天然的蛋白结构。

关键词：鲢鱼、氧化、蛋白功能性、冷冻保护剂、抗氧化剂、冷冻保藏.

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ABBRIVIATIONS USED

MPI	Myofibrillar protein isolate
IOS	iron-catalyzed oxidation system
PS	protein solubility
GS	gel strength
PUFAs	poly-unsaturated fatty acids
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid
ALA	linolenic acid
LA	linoleic
AA	arachidonic acids
WHC	Water Holding Capacity
OHC	Oil Holding Capacity
EC	emulsifying capacity
FAO	food and agricultural organization
S	cryoprotectants (sucrose and sorbitol)
A	ascorbate
E	α-tocopherol
d	day
t	time
WF	whole fish
FM	fish mince
SDS-PAGE	Sodium dodecyl sulfatepolyacrylamide gel electrophoresis
DSC	*Differential scanning calorimetry*
RCS	mechanical refrigeration system
BSA	bovine serum album

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

China is the largest producer of freshwater fish in the world, (Jorge and others 2009; Ministry of agriculture 2006). Among cultivated fresh water fish, silver carp *(Hypophthalmichthys molitrix)* contribute noticeably to the total catches. With its tremendous growth rate, is expanding quickly in China and other countries such as, Bangladesh, India, Iran, and Russian Federation (FAO 2007). It is mainly marketed for fresh consumption, however its consumption is still low in spite of being the cheapest fish owing to its muddy flavor and the muscle embedded tiny bones. Demand for processing and storage of silver carp are the best optional, since silver carp meat can be used as an excellent raw material for surimi production and for incorporating in highly accepted products (Daley and Deng 1978).

Fish Myofibrillar protein isolate (MPI) is a kind of protein ingredient which is prepared from fish muscle, without retaining the original shape of the muscle. It is generally not consumed directly but is useful in restructured meat and other value-added products (Srinivasan and Xiong 1996). MPI are of great importance to the functional properties of meat, in particular protein solubility (PS), water holding capacity (WHC), oil holding capacity (OHC), emulsifying capacity (EC), and gel strength (GS). However a great challenge of this protein ingredient is oxidation which is known to be the primary cause of protein functionality alteration in muscle foods, as, it affects textural attributes of fish products (Xiong and Decker 1995). Fish muscles are susceptible to oxidative reactions due to their high concentrations of oxidation catalysts (iron and myoglobin) and unsaturated lipids (Asghar and others 1988; Srinivasan and Xiong 1996). Silver carp contains high amount of poly-unsaturated fatty acids (PUFAs) including eicosapentaenoic (EPA), docosahexaenoic (DHA), linolenic (ALA), linoleic (LA) and arachidonic acids (AA) (Taskaya and others 2009) than pork which is rich in saturated fatty acids (Park and others 2006), unfortunately PUFAs are prone to oxidation producing reactive oxygen species capable of modifying protein structure. Also it is well documented that lipid oxidation leads to modification of proteins in biological systems (Tokur and Korkmaz 2007b; Xiong and others 1993; Fu and others 2009). Although the effect of protein oxidation has not been reported in silver carp, it has been reported in pork muscles (Park and others 2006) and chicken muscles (Liu and Xiong 2000).Those reports have focused mainly on oxidation of muscles protein in mammals which are known to have relatively low level of PUFAS compared to those found in fish. To exploit silver carp, there is still a great demand to study protein oxidation as a complement to the

research done on lipid oxidation on the same fish species (Fu and others 2009). In addition due to high concentrations of oxidation catalysts (iron and myoglobin), fatty acids, other lipids, oxidized lipids in silver carp muscles, are likely to initiate the change in functional properties of proteins (Taskaya and others 2009; Asghar and others 1988). Furthermore, fish processing such as mincing and grinding could further increase oxidative reactions in muscle foods by mixing oxidation catalysts with lipids and oxygen (Smith 1987).

Iron is an important catalyst, well suited to catalyze redox, as many published works show that the Fe^{+2}/H_2O_2 mixture is determined as the most efficient systems among the different metal catalyzed oxidation systems (Martinaud and others 1997; Mercier and others 2004; Nagasawa and others 2001). Apart from lipid oxidation which has been lengthily studied, limited or no research work has been done on the effect of protein oxidation of silver carp proteins during processing and storage.

Salting of fish is an old and traditional method to improve shelf life. The importance of it mainly is adjusting ionic strength which has a great influence on protein functionality such as solubility, gel development to mention a few. A change of ionic strength influences muscle characteristics including heat-induced gelation, thermal properties, water binding capacity and emulsification properties (Stanley and others 1994). The structure of a protein and its functionality can also be modulated by environmental factors, especially temperature and pH (Myers, 1988; Supawan and Jae 2007). By controlling environmental conditions, it is possible to gain more insight into how the structure of a protein dictates its function. The salt concentration affects the stability and denaturation of proteins. The addition of sodium chloride above the isoelectric point of proteins (pI), in meat systems, causes swelling and an increase of water holding capacity (Kristin and others 2002). The salt ions are believed to cause weakening of the interaction between oppositely charged side chains, which results in swelling. A measurable increase in WHC has been observed by the addition of more than 1% salt NaCl, maximum swelling has been estimated at approximately 5% but, at higher concentrations, the myofibrilar proteins rapidly loose water through the salting-out process (Kristin et al., 2002).

The isoelectric point of myofibrillar proteins is pH ~ 5 and increasing or decreasing pH from this value results in a more negative or positive charge of the myofibrillar proteins, respectively (Bertram and others 2004). This causes an increased repulsion of the myofilaments and thereby enlarged myofilament lattice, which was demonstrated by Rome, (1967) using X-ray diffraction. Likewise, the charge of the

myofibrillar proteins are altered by ionic strength as the binding of negatively charged ions leads to a shift in pI (Hamm, 1986). It has been demonstrated that ionic strength affects the degree of swelling of the myofibrillar protein (Bertram et al., 2004; Offer and Trinick, 1983). In addition, it has also been shown that pH and ionic strength affect the microstructure of gels (Bertram et al., 2004; Hermansson and others 1986).

Frozen storage is an important method for a long term preservation and storage and it is widely used for keeping the raw materials in good condition before use in any value added products. It is also the only large scale preservation method that facilitates exports and minimizes variations in supply of raw fish (Persson and Londahl, 1993). The quality of frozen fish has been studied and variations in the stability of fish species stored frozen under the same conditions have been observed (Stamatia and others 1997). It is generally accepted that there are two main problems associated with frozen storage of fish: hydrolysis and oxidation of lipids and protein denaturation (Stamatia et al., 1997; Verma and others 1995). Various factors, such as the freezing temperature, the rate of freezing, vacuum packaging or packaging materials, can affect frozen fish quality, and these have been studied (Stamatia et al., 1997). Frozen fish are often stored in the form of fillet; however, filleting operations can affect frozen fish quality (Ciarlo and others 1985; Verma et al., 1995).

Storage of fish at low temperature sometimes causes insolubilization of myofibrillar proteins and loss of its functionality such as PS, GS and, WHC) (Ramirez and others 2000). Fish muscle proteins are less stable to frozen storage compared to avian and mammal muscle proteins due to their high concentrations of oxidation catalysts (iron and myoglobin) and unsaturated lipids (Asghar and others 1988; Srinivasan and Xiong 1996). In addition, the differences could be attributed to the fact that fish are coldblooded animals while avian and mammals are warm-blooded (Kamath and others 1992; MacDonald and Lanier, 1991).Myofibrillar protein instability during frozen storage has been associated with changes in the stability of fish myosin (the major contractile protein of skeletal muscle), and this phenomenon is dependent on habitat temperature, with myosin from cold-water species being less stable than myosin from warm-water species (Davies and others 1994; Howell and others 1991; Misima and others 1990).

There are three theories which describe alterations in the protein microenvironment that cause protein denaturation during freezing and frozen storage, such as an increase in solute concentration, dehydration of the cell, and autoxidative

changes that alter the balance of protein–protein and protein–water interactions. As freezing progresses, proteins are exposed to increased ionic strength in the non frozen aqueous phase that leads to extensive modification of protein native structure (Jittinandana and others 2003; Lin and Park, 1998). In a dehydrated state, protein–water interactions in tissue are disrupted, and protein molecules are exposed to an organic environment that is less polar than water. These changes result in increased exposure of hydrophobic side chains and, therefore, changes in protein conformation (Franks, 1995; Jittinandana and others 2003).

Moreover, frozen stability of fish muscle proteins varies among species (Hastings and others 1985). Silver carp contains high amount of poly-unsaturated fatty acids (PUFAs) (Taskaya and others 2009; Mlyuka and others 2010), unfortunately PUFAs are prone to oxidation producing reactive oxygen species capable of modifying protein structure. Also it is well documented that the myofibrillar protein of many fish species may be altered by their interaction with different type of lipids or lipid oxidation products during frozen storage (Saeed and others 1999). Thus, during frozen storage the products of lipid oxidation render the fish tissue proteins into harder, more elastic and insoluble complexes (Takama and others 1972). In addition, there is a loss of Specific amino acids such as cysteine, lysine, histidine and methionine, as well as damage to other pigmented proteins such as cytochrome C and haemoglobin (Roubal and Tappel, 1966).

Cryoprotectants improve quality and extend shelf life of frozen foods by preventing deleterious changes in myofibrillar proteins caused by freezing, frozen storage, and thawing (Jittinandana and others, 2003). Among many cryoprotectants used in frozen foods, a sucrose/sorbitol mixture is commercially used in minced fish products due to availability and low cost (Jittinandana and others, 2003; Lee, 1984).

Inhibition of protein and lipid oxidation in muscle protein can be achieved, among other means, by incorporating water and/or lipid-soluble antioxidants, or their combinations (Srinivasan and others 1996).

Therefore, cryoprotectants and antioxidants have been mainly used during frozen storage to improve functional properties, avoid lipid oxidation development, inhibit dripping loss during defrosting, and to prolong storage time (Pazos and others 2005)

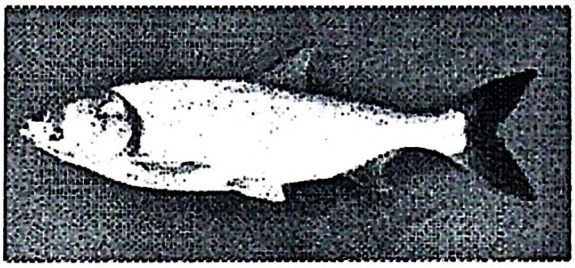
Silver carp has acquired great attention because of its increasing farming production and application in the surimi-product commercialization in Asia particularly China. Increased Silver carp production requires frozen storage to facilitate distribution and inventory control.

1.2 LITERATURE REVIEW

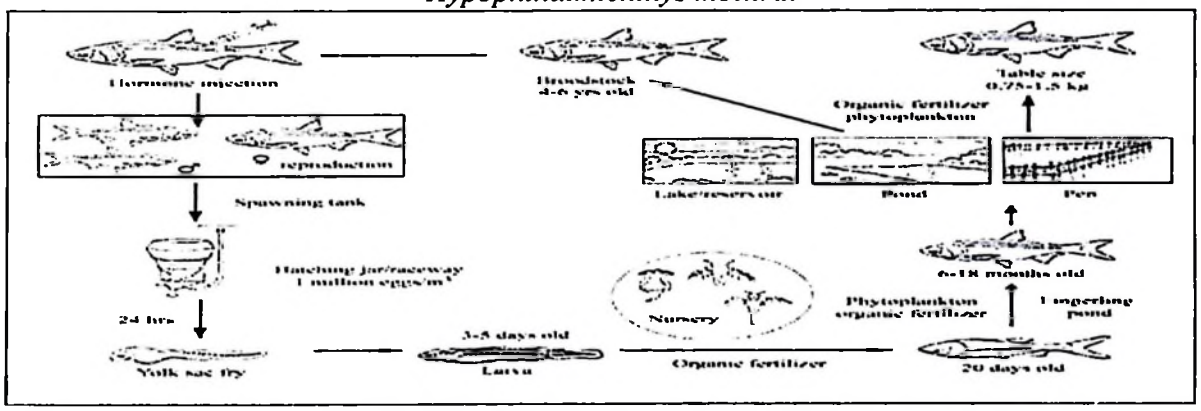
1.2.1 Silver carp Production and Consumption

1.2.1.1 Production

Silver carp is a freshwater species living in temperate conditions (6-28 °C) and its natural distribution is in Asia (Qingwen and others 1995; FAO, 2007; Yujun, 1981; Ministry of agriculture, 2006). This species requires static or slow-flowing water, as found in impoundments or the backwaters of large rivers.



Hypophthalmichthys molitrix



Adopted from Qingwen and others 1995

Figure 1.2. 1-Production cycle of *Hypophthalmichthys molitrix*

1.2.1.2 Handling and processing

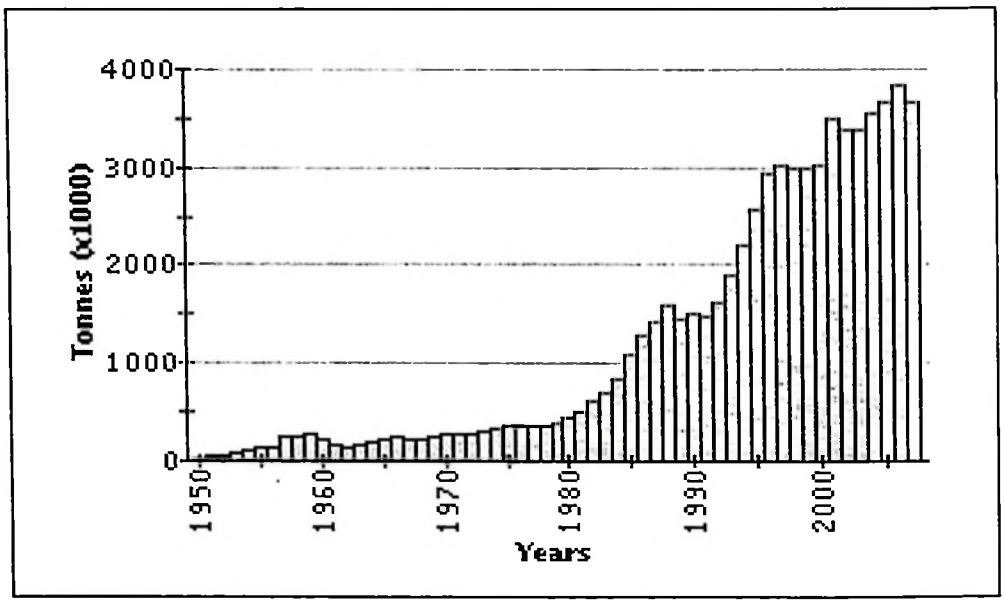
Silver carp are normally bought live, based on traditional consumption patterns in China. It is therefore essential to keep them alive from harvesting to marketing. Trucks and boats containing water are basically used as transportation tools in most areas.

1.2.1.3 Production costs

The production costs for silver carp vary from country to country (and even place to place) and the scale of operations. The major factors are the cost of labor, culture facilities, water, seed, feed (fertilizer), power, and transport.

1.2.1.4 Production statistics

The largest producer of silver carp through aquaculture is China, while India and Bangladesh are also major producers of this species. Significant amounts of silver carp are also raised in Iran, the Russian Federation and Cuba (Qingwen et al., 1995; FAO, 2007; Yujun, 1981; Ministry of agriculture, 2006).



Source:FAO, 2007

Figure1. 2 .2-Global aquaculture production of *Hypophthalmichthys molitrix*

1.2.1.5 Consumption

Hypophthalmichthys molitrix is generally cultured and consumed locally alive or fresh in most of the producing countries. No information is available on international trade (Jorge and others 2009). The market price in China for this species is relatively low compared to most other species, normally 8-10 Yuan/kg .There are no specific market regulations for silver carp; it is treated the same as most fish commodities in the markets.

1.2.1.6 Status and trends

Silver carp is a native species in China and Eastern Siberia, but has been introduced to more than 88 other countries for aquaculture and its use in controlling algal blooms. It is not only utilized as human food but also appreciated by its ability to clean reservoirs and other waters from clogging algae.

The culture of silver carp has expanded steadily over the past decade, rising from 1.9 million tons in 1993 to 4.1 million tons in 2003(Jorge et al., 2009; Anony, 2002; FAO, 2007). Further expansion is expected as its production costs are lower than most other cultured species since there is no need to provide supplementary formulated feed, and most ordinary people can afford to consume it regularly.

In order to keep the product longer and further extend the markets instead of just consuming it fresh, processing technologies need to be developed.

1.2.1.7 Application of silver carp and silver carp products

Apart from application of this fish species on surimi production, many research works concerning this fish has been done, these include research on composition studies (Vujkovic and others 1999) and its employment as a source of protein hydrolysates (Dong and others 2008) and commercial products (Liu and others 1991; Fan and others 2008).In addition, Yuan and others 2005,studied the gel forming capacity of this species with respect to different catching season and the presence of different binding agents on gel-forming capacity (Uresti and others 2004) as well as making comparisons to other fish species (Luo and others 2001). More than that, Taskaya and others (2009) studied the compositional Characteristics of materials recovered from silver carp waste. More over (Afsaneh and others 2010) studied the chemical changes in silver carp minced muscle during frozen storage.

1.2.2 Myofibrillar Proteins

The myofibrillar proteins are essential for maintaining the binding properties of comminuted meat and fish products, such as sausages and kamaboko (is a variety of Japanese processed seafood products, made from surimi, in which various white fish are pureed, formed into distinctive loaves, and then steamed until fully cooked and firm in texture), English names for kamaboko are *fish paste*, *fish loaf*, *fish cake*, and *fish sausage* (Tsuji, 1980). Myofibrillar proteins are also partially responsible for fat and water binding capacities and emulsifying ability, their alteration could have an adverse effect on the physical properties of the final products. Myosin, the major constituent of

myofibrils, is of technological significance, due to its participation in the rheological changes that take place during the storage and processing of meat and fish products (Rodger and others 1984). Myosin is the most abundant single muscle protein in fish muscle, comprising about 50% of the myofibrillar contractile proteins and around 40% of the total protein (Kristinsson and Rasco, 2000).

1.2.2.1 The impact of oxidation on myofibrillar protein functionality

Protein functionality is generally defined as any property of a food or food ingredient except its nutritional property that influence its utilization. Also Xiong (1996) defined functionality as the behavior of protein molecules, on a macroscopic scale, before, during and after food processing which determines the quality of final products. Functional properties are regarded as "intermediate" properties in the sense that they are dictated by physicochemical properties of proteins, but they play a critical role in producing desirable product characteristics as shown: physicochemical properties →functionality → product quality. This relationship describes those alterations of protein functionality by oxidative stress result from physicochemical changes of the protein. Hence, to elucidate the impact of oxidation on muscle protein functionality, it is important to define chemical and physical changes involved in the initial stage of oxidative attack. Protein oxidation is accompanied by a variety of physicochemical modifications, including amino acid destruction, unfolding and denaturation of protein molecules, peptide chain scission and insolubilization of protein. When free radicals are involved, molecular polymerization and formation of lipid-protein complexes can occur.

1.3 Justification

Fish species differ in their susceptibility to protein denaturation and aggregation during frozen storage, Silver carp deserve detail research on this aspect due to its importance as new raw materials for surimi production and its commercialization. The study of the influence of frozen storage and fatty acids oxidation on silver carp myofibrillar protein functionality is geared by its unique role on particular muscle food system. The great concern is on physico-chemical properties of silver carp myofibrillar proteins, described as functional properties which affect the ultimate texture of finished muscle food products. In addition, fish products are stored and marketed in frozen state, as freezing and frozen storage are important methods of preserving fish and fishery products. Although undesirable changes such as microbial aspects and other chemical alterations are controlled during frozen storage, changes do occur in the quality of protein which in turn has impacts on functionality of the proteins. Thus, the present work focused

mainly on the changes in proteins and lipids of silver carp beheaded fish, fish mince and MPI functionality during frozen storage. This study can lead to better utilization of low priced fishes in the preparation of fishery products, in which functionality of protein is of great significance.

1.4 OBJECTIVES OF THE STUDY

- ❖ The influence of frozen storage and fatty acids oxidation on silver carp myofibrillar protein functionality.

1.4.1 Specific objectives

1. Isolation of silver carp myofibrillar protein for functionality study.
2. Investigate the impacts of Iron oxidation system on myofibrillar protein functionality.
3. Determine the effects of cryoprotectants on the functional properties of the myofibrillar protein isolate
4. Determine the effects of antioxidants on the functional properties of the myofibrillar protein isolate.
5. Examine the influence of fatty acids oxidation on protein functionality for frozen stored silver carp myofibrillar protein.

2.0 MATERIALS AND METHODS

2.1 Materials

A live silver carp fish (Hypophthalmichthys molitrix) was purchased from a local market at Wuxi (Jiangsu Province, China), Preparation of the sample was carried out at 0-4°C microenvironment controlled by ice crystals in cold room set at 4°C. All chemicals used were of analytical grade.

2.2 Methods

2.2.1 Preparation of myofibrillar protein isolate

Preparation of silver carp myofibrillar proteins was done according to (Park and others 2006), with a slight modification, centrifugation was adjusted to 5000g for 15 min to favor precipitation of fish MPI. The protein concentration in MPI was determined by the Biuret method (Gornall and others 1949) using bovine serum albumin as the standard.

2.2.2 Preparation of sample for storage

Preparation of MPI for frozen storage, consisted of myofibrillar (4% final concentration), 8% cryoprotectants (4% sucrose and 4% sorbitol), and antioxidants (0.2% ascorbate, 0.2% α-tocopherol or their combination), and for some treatments ionic strength was adjusted to 0.6M NaCl (2.5%) (to solubilise myofibrillar protein). The pH was adjusted to 6.5 with 0.1N HCl prior to frozen storage to simulate the pH condition in unprocessed fish.MPI were divided into groups, one group was stored with either cryoprotectants (MPI+S) or a mixture of cryoprotectants and ascorbate (MPI +S+A) or a mixture of cryoprotectants and α-tocopherol (MPI +S+E) or mixtures of cryoprotectants,0.1%ascorbate,0.1% α-tocopherol (MPI+S+A+E) or with adjusted to 0.6M NaCl (MPI + 0.6 NaCl).The mixtures were kept in a beaker under ice and were thoroughly mixed using glass rod. The control was prepared in the same manner, except cryoprotectants and antioxidants and additional of sodium chloride was not incorporated into the sample. Then the samples were packed in a polyethylene bag and sealed under air. All samples were kept at refrigeration temperature for six hours to equilibrate before frozen storage at -18°C for 90 days. At definite time intervals 3,7,14 30, 60 and 90days, samples were removed, thawed with running water (26 – 28°C) to obtain the core temperature of 0 – 2 °C and then analyses were carried out.

The whole fish were washed and beheaded, followed by packing individually in polyethylene bags and was buried in ice in a tape water trough with drainage provision. The package was then stored in a refrigeration cabinet for six hours soon after preparation

for frozen storage for resolution of rigor mortis. At the end of storage period, the fish were filleted and skinned by hand. Dorsal fillet were used for MP isolation of which was subjected to different biochemical tests. No washing was applied prior to MP isolation in order to avoid any variability due to absorbed or melted water. The mean weight of the skinned fillets was 72 ± 10 g (average ± S.D.) per fish. For fish mince storage, fish fillets were minced with knife, followed by packing in polyethylene bags 100g in each preparation and then were sealed under air. Finally the packages were kept in the fridge for six hours for resolution of rigor mortis prior-to frozen storage. Also were sampled at definite time intervals, 3,7,14 30, 60 and 90days for analyses. Thawing was done using w running tape water (26 – 28 °C) to obtain the core temperature of 0 – 2 °C and then were subjected to analyses.

2.2.3 Proximate analysis of silver carp fillet

The moisture content of the fish fillet was determined by evaporation method. The sample was dried in an oven set at 105°C for 24 h. Ash content was performed by incinerating samples in a muffle furnace at 550℃. The lipid content was determined by extracting the sample in Büchi extraction system B-811, Labortechnik, Deutschland with petroleum ether and then by weighing after evaporating the petroleum ether. The protein contents were determined by Kjeldahl method using Buchi distillation unit K-350 (BÜCHI Labortechnik Deutschland) according to (AOAC, 1999). Proximate analyses are expressed as percent (wet weight basis).

2.2.4 Crude fat content and fatty acid analysis

The extraction of crude fat from silver carp was done according to China national method for determination of fat in foods (GB/T5009.6-2003). After extraction, the solvent was evaporated at 25 ° C in a water bath put in a fume hood chamber. The crude fat was dried in an oven at 105 ° C for 2h, desiccated for 30 min, and weighed.

To determine the fatty acid profile in the crude fat, aliquots of approximately 34.4 mg of extracted crude fat were treated according to procedures adopted from Park and others (2006). Finally supernatant, 1μLof fatty acid methyl esters were injected into gas chromatography, GC-14B (Shimadzu, Japan) equipped with a CP-WAX capillary column (30m x 0.32mm inner diameter) and flame detector, for analysis. The fatty acids in the sample were identified using methyl ester standard mixtures and were confirmed by GC-mass. The nitrogen carrier gas flow rate was 3.0 mL per min. The injector and detector were set at 250 °C. The initial oven temperature was 120 °C, which was held for 3 min

and then increased at a rate of 10 °C per min to 190 °C. The final temperature was 220°C at a rate of 2 °C per min held for 15 min. In the injector, a split valve was set at a 60:1 ratio.

2.2.5 Chemical induction of MPI oxidation

MPI (20mg/mL) was homogenized in 100 mL of 50 mM sodium phosphate buffer (pH 7.0), using a homogenizer (Ultra Turrax (IKA® T 18 basic, Germany) for 1 min at relatively low speed (6500 rpm). Ferrous sulphate (1 mM) and hydrogen peroxide (10 mM) were added to homogenate. The mixtures were incubated for 0, 3, 6, 9,12,18,21 and 24 h at 4°C in a freezer cabinet, and the reaction was stopped by adding butylated hydroxytolune (BHT, to 0.02% final concentration). The mixture was analyzed immediately for protein oxidation.

2.2.6 Determination of Protein carbonyl content

The protein carbonyl contents in MPI incubated at 0, 3, 6, 9, 12,15,18,21 and 24h was detected by reactivity with 2, 4 dinitrophenylhydrazine (DNPH) to form protein hydrazones as described by Levine and others (1994);Tokur and Korkmaz (2007a). Finally, all samples were centrifuged to remove any insoluble materials. The concentration of DNPH was determined at 370 nm against the blank of 1 mL guanidine solution, and molar absorption coefficient of 22,000 $M^{-1}cm^{-1}$ was used to estimate protein carbonyls. Protein concentration was determined at 280 nm of absorbance in the samplesusing BSA in 6 M guanidine as standard. The results were expressed as nmol carbonyl /mg protein.

2.2.7 Determination of sulfhydryl (-SH) groups

Total sulfhydryl contents were determined using 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) according to a modified technique proposed by (Chen and others 1989). The resulting mixture were incubated at 25°C for 45 min and were then centrifuged 3 min at 1940 g using Sigma laboratory centrifuge (3K15,Germany) before reading absorbance of the supernatant at 412nm and the -SH content was calculated using a molar extinction coefficient of 13,600 $M^{-1}cm^{-1}$. Results were expressed in micromoles of -SH per mg of MPI.

2.2.8 Determination of dityrosine formation

Bityrosine formation was estimated by the method of Davies (1987), with a slight modification by Morzel and others (2006). Dityrosine content was estimated by

fluorescent measurement at 420 nm (band width = 10 nm) after excitation at 325 nm (band width = 10 nm), using a spectrofluorophotometer fitted with a 150 W Xenon lamp (Hitachi ltd 650-60, Tokyo, Japan). Corrected fluorescence was obtained by dividing measured fluorescence by protein concentration. The results were expressed in arbitrary units.

2.2.9 Sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE is a technique in which proteins are placed in an acrylamide gel where the anode and cathode are located at opposite ends of the gel. The proteins may then be separated by charge and size. Electrophoresis is normally operated at high pH when the proteins take on a net negative charge. When the surface active agent, sodium dodecyl sulfonate (SDS), is added to the sample, it acts as a denaturant and a solubilizing agent, unfolding the peptide chains and converting them to a rod-like shape. The SDS binds to the proteins and cancels out the intrinsic charge of the proteins giving them the charge of SDS (Thorarinsdottir and others 2002; Pomeranz and Meloan 1987). The separation by electrophoresis in the SDS-PAGE system is mainly based on molecular weight, but not charge. The technique has proven to be an excellent tool for the determination of molecular weight and for analyzing the number of subunits in the protein molecule. SDS-PAGE can be used, both as a qualitative, and quantitative, method in the identification of different proteins. SDS-PAGE has been used for identification of different muscle proteins and their subunits in fresh muscle and also to estimate the effects of storage and processing on the stability of proteins (Thorarinsdottir others 2002; Garcia and others 1997; Bechtel and Parrish 1983).

SDS Gel Electrophoresis was performed according to the standard procedure (Laemmli, 1970). SDS-PAGE was used to monitor oxidation induced polymerization or fragmentation of the oxidized MPI. The stacking and resolving gels contained 5 and 12% acrylamide, respectively. Samples subjected to SDS-PAGE were treated with or without β-mercaptoethanol, a disulfide-breaking agent. The separating gel was run at a constant current of 30mA for 2 h, using a BIORAD electrophoresis apparatus (Powerpac Basic, USA).

2.2.10 Gel-permeation chromatography (GPC)

GPC method was used to monitor oxidation induced polymerization or fragmentation of the oxidized MPI since the properties of oxidized proteins are so varied, it is impossible to describe using one single method setting out precisely, the results of oxidation. A Shodex- KW804 GPC Column with a protein exclusion limit of about 200

kDa was used for GPC. The column was hooked to high-performance liquid chromatography (HPLC) with a manual injector, waters pumps, and UV (220nm and 280nm) waters detector. Breeze software was used for data acquisition and processing. The GP chromatography was carried out at elution rate 1mL/min with phosphate buffer solution (0.05M PBS) in 0.5 M NaCl (pH7.0) as an eluent. Since GPC is a relative method, calibration was done using standard with known molecular weights. The column was calibrated with 8 protein standards covering the molecular weight range from 1kDa to 660kDa.Both MPI samples untreated and treated with IOS were diluted with 0.5M NaCl, 50mM phosphate buffer, pH 7.0, to a concentration of about 1mg/mL and centrifuged at 12000 g for 3min, 20μL of the supernatant of each sample was loaded on the column.

2.2.11 Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed on a Perkin-Elmer Instruments (Perkin-Elmer, Pyris 1, USA). A liquid nitrogen-cooling accessory was used to reduce the cell temperature; also, a mechanical refrigeration system (RCS) was applied using ice. The instrument was temperature-calibrated, using milli-Q deionised water and indium. Enthalpy was calibrated with indium. Empty pan was used as reference. For the determination of the denaturation properties, MPI weighing 6.8- 10.8 mg (accuracy of ±0.01 mg) were sealed in hermetic aluminium pans and scanned at a heating rate of 10°C/min over the range 20–90°C. The denaturation temperature (Td) and enthalpy (ΔHd) were considered as those corresponding to the maximum peak and to the area of the endothermic peak, respectively, also peak temperatures corresponding to transition temperatures for myosin and actin were recorded.

2.2.12 Peroxide value

The peroxide value (PV) was determined according to (AOAC, 1998) Official Method Cd 8-53 Analysis was carried out in duplicates.

2.2.13 TBA test

Lipid oxidation was evaluated by production of thiobarbutiric acid reactive substances (TBARs) according to the method of Yu and Sinnhuber (1957). 3 mL of 1 % (w/v) 2- thiobarbutiric acid in 0.3%NaOH and 5ml of 2.5 % (w/v) trichloroacetic acid were added to 0.4g of frozen samples. The samples were then incubated in a boiling water bath for 30 min, cooled and extracted with 5ml of chloroform. After clarifying the pink chromagen, the color intensity of lower layer was measured at 532 nm against a

blank. The concentrations of TBARs in samples were calculated by using the following equation;

$$TBARs(^{mg}/_{Kg}) = (^{A532}/_{Ws}) x 9.48 \quad \textbf{Eq.1.}$$

Where by A_{532}= absorbance read at 532 and Ws =weight of sample (g)

The results were expressed as mg malonaldehyde (MDA) per kg fish muscle.

2.2.14 Total Amino Acid Analysis

The sample prior to analysis was hydrolyzed with 6MHCl (final concentration 0.4%protein).Then, was analyzed with an Agilent HP1100 amino acid analyzer (Agilent Co., Palo Alto, CA) equipped with a C_{18} column (4.6 mm × 125 mm) for amino acid separation. Precolumn reaction with phthalic dicarboxaldehyde (OPA) yielded amino acid derivatives. The concentrations of the specific amino acids were determined from their respective absorption intensities, which were calibrated to the known concentrations of amino acid standards.

2.2.15 Free Amino Acid Analysis

The method described byWu and others 1995 was used with slight modifications. Samples were precipitated with 10% cold trichloroacetic acid for 1.5 h and then centrifuged at 12000*g* for 10 min. The solution was passed through a microfiltration membrane (0.45 μm). The filtrate was subjected to RP-HPLC analysis (Agilent HP1100) after precolumn derivatizing with OPA as indicated above.

2.2.16 Solubility of Silver carp MPI

Sample (0.5 g MPI) was homogenized in 10 mL of 0.5 M NaCl in 50 mM pH 7.0 phosphate buffer using Ultra Turrax (IKA® T 18 basic, Germany); the homogenate was centrifuged at 5000g for 15 min at 4°C using Sigma laboratory centrifuge (3K15,Germany). The supernatant was diluted with 0.5M NaCl and protein determination was performed using Biuret method. The solubility was expressed in percent soluble MPI.

$$\%solubility = (\frac{protein\ concentrtion\ in\ the\ supernatant}{original\ protein\ concentration})X100 \quad \textbf{Eq.2.}$$

2.2.17 Water holding capacity

WHC was determined by centrifugation method described by CObb III and Hyder, (1972). The difference between the initial volume of distilled water added to the MPI sample and the volume of the supernatant was determined, and the results were reported as mL of water absorbed per g of MPI.

2.2.18 Oil holding capacity

OHC was measured as the volume of edible oil held by 50 mg of MPI (CObb III and Hyder, 1972). The volume of oil separated from the MPI gel through centrifugation, was reported as the amount of oil in mL absorbed per g of MPI.

2.2.19 Emulsifying capacity

EC was measured using the procedure described by Rakesh and Metz (1973). A 0.5 g MPI and 30 mL of soy bean oil were added to 60 mL of NaCl solution (30 g/L) and mixed using a homogenizer (Ultra Turrax (IKA® T 18, Germany) at 9500 rpm for 30 min. After this period, another 30 mL of oil were added within 1.5 min and mixed for a further 30 s. The mixture was transferred to centrifuge tubes, held in a water-bath at 85 ° C for 15 min, and then centrifuged at 3000 g for 30 min.

Emulsifying capacity was calculated using Eq.3:

$$EC\ (oil\ emulsified) = \frac{VA - VR}{WS} \quad \textbf{Eq.3}$$

Where VA is the volume of oil added to form an emulsion, VR is the volume of oil released after centrifugation and WS is the weight of the sample MPI.

2.2.20 Gel preparation and gel strength measurement

Gel preparation and gel strength measurement were performed using procedure described by Srinivasan and Xiong (1996) with a slight modification. Protein suspensions (40mg/mL) in 0.6M NaCl, 20mMphosphate buffer (pH 6.0) were used .The final pH of all protein suspensions was maintained at 6.0 and equilibrated for 24 h at 5 ° C before heating in a water bath at 40°C for 20 min in 16.5 (inside diameter) × 50 (length) mm

glass vials, covered with aluminum foil. Gels were penetrated with a flat-faced stainless steel probe (10 mm dia.) attached to a Model TAXT2 texture analyzer Systems (Stable Micro Systems Ltd., Surry, UK) at a crosshead speed of 0.5 mm/s. The penetration force required to rupture the gels, was expressed as gram force (gf).

3.0 RESULTS AND DISCUSSION

3.1 Susceptibility of silver carp myofibrillar protein to iron-catalyzed oxidation and resulting functionality Changes

3.1.1 Proximate Analysis of Silver Carp fillet

Silver carp fillet mainly composed of water, 78.1± 0.2% moisture content, after water it composed of about 18.2 ±0.52% protein and relatively small amount of fat measuring 1.2±0.12% since it is a lean fish. 1.09±33% ash content was obtained by incinerating fish fillet in a muffle furnace at 550°C.

3.1.2 Crude fat content and fatty acid analysis

The total fat content of silver carp MPI was 0.6%, it is mainly composed of unsaturated fatty acids (Table1) of which about 34% constituted by EPA, (20:5ω-3), DHA, (22:6ω-3), and ALA, (18: 3ω-3) as the main ω-3 PUFAs, as well as LA, (18:2ω-6) and AA, (20:4ω-6), the main ω-6 PUFAs in fish fillets similar to that reported by Taskaya and others (2009), unfortunately PUFAs are prone to oxidation producing reactive oxygen species capable of modifying protein structure.

Table 1 – Fatty Acid Profile in Silver carp MPIs

Fatty acid	Carbon number	Relative quantity (%)
myristic acid	C14:0	1.34
palmitic acid	C16:0)	21.19
palmitoleic acid	C16:1	7.59
stearic acid	C18:0	10.55
oleic acid	C18:1	25.04
linoleic acid	C18:2	2.47
linolenic acid	C18:3	2.62
arachidic acid	C20:0	0.32
eicosenoic Acid	C20:1	2.27
arachidonic acid	C20:4	5.99
eicosapentaenoic Acid	C20:5	9.67
Docosahexaenoic	C22:6	10.94

3.1.3 Protein carbonyl content

A significant increase *($P < 0.05$)* in the carbonyl groups was detected in MPI incubated in IOS for 24h (Figure 3.1). In this study, a control was used at 0 h oxidation only, purposely to determine the initial level of protein oxidation, according to (Smith, 1987), muscle foods subjected to comminuting processes, such as mechanical deboning, show decreased protein functionality which could be due, in part, to the oxidation of proteins. Thus, throughout the study, control was used at initial stage of oxidation to determine the effect of mechanical deboning since no enzymes inhibitors were employed in the whole process apart from working at low temperature. The initial level of carbonyl was 14.32 nmol of DNPH per mg of protein; this value was 4.4 times higher than those reported by Mercier and others (2004) in beef homogenate oxidized with Fe^{+2}/H_2O_2 oxidation system. Meanwhile carbonyl content was 17.09 nmol/mg proteins at 0h of incubation in IOS (Figure3.1), which increased linearly reaching a maximum protein carbonyl of 24.44nmol/mg at 24h of oxidation, the trends observed are fairly consistent with that reported by Morzel and others (2006). In contrast to that reported by Tokur and Korkmaz (2007a) on oxidation of grey mullet by Fenton oxidation system, this difference reveals that, the response of the fish and fish products to oxidation of protein, by IOS, can vary, depending on fish species (Tokur and Korkmaz, 2007b). The significant increase in carbonyl content *($P<0.05$)*, at 24h of incubation of MPI in IOS suggests that, silver carp MPI is highly susceptible to iron oxidation during processing either for storage or preparing for value added products using fish meat as a raw materials without antioxidants.

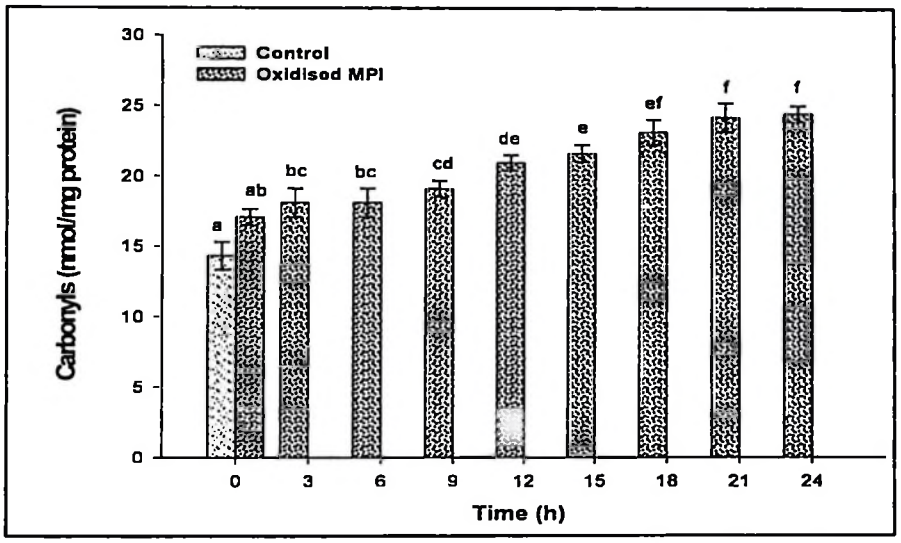


Figure 3.1-Effect of different iron oxidation (Fe^{+2}/H_2O_2) time on carbonyl content of fish myofibrillar proteins. Carbonyl content of fresh myofibrils is included as control. Groups with different letters (a, b, c, d, f) are significantly different (*P<0.05*).

3.1.4 Effect of iron-catalyzed oxidation system on the total sulfhydryl content of silver MPI

The total sulfhydryl content (total -SHs) of silver carp MPI decreased significantly *(P<0.05)* with oxidation time as a result of IOS (Figure 3.2). The decrease in -SH content was reported to be due to the formation of disulfide bonds through oxidation of -SH groups or disulfide interchanges (Hayakawa and Nakai 1985). The implication is, therefore, those disulfides bonds are involved in an iron induced aggregation of fish MPI as was confirmed by GPC results (Table 2 & Figure 3.5). Thiols are sensitive to oxidation and are generally believed to be a major target for oxidation in proteins (Stadtman and Levine 2003).

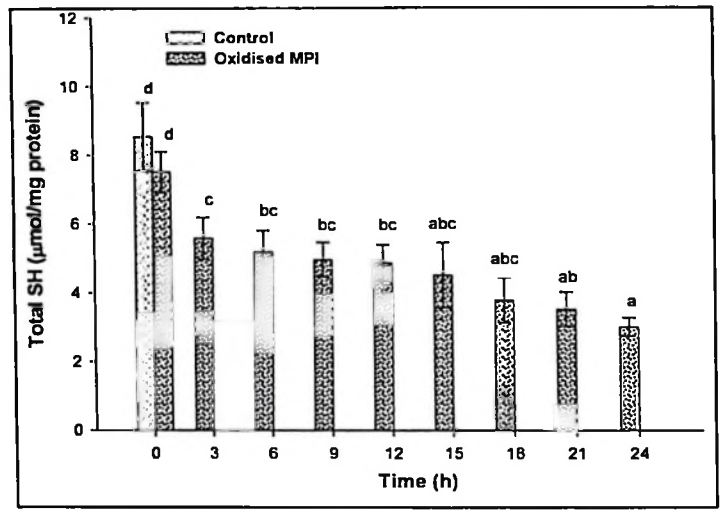


Figure 3.2 -Effect of different iron oxidation time on total sulfhydryls content of fish myofibrillar proteins. Fresh myofibrils were included as control. Groups with different letters (a, b, c, d) are significantly different (*P<0.05*).

3.1.5 Determination of dityrosine formation

Oxidative damage, particular to proteins, has been widely postulated to be a major causative factor in the loss of functional properties during oxidation. Tyrosine oxidation products can be used as markers for oxidative stress invitro due to the formation of dityrosine upon oxidation which has distinct fluorescent properties as described to be a useful indicator for protein modification (Davies 1987). Thus in this study, dityrosine formations increased significantly at 24h *(P<0.05)* for MPI incubated in IOS (Figure 3.3) indicating a progress increase of oxidation degree with respect of oxidation time. This observation suggests that, MPI underwent protein modification which involved amino acid side chains. Similar observations had been reported by Morzel and others (2006). The observed decline of MPI functionality (Table 3) is assumed to be due to dityrosine formation, disulfide bonds formed through oxidation of cysteine thiol groups and reactions between protein carbonyls (formed through oxidation of protein side chains) and the α-amino group of lysine side chains (Stadtman and Levine 2003).

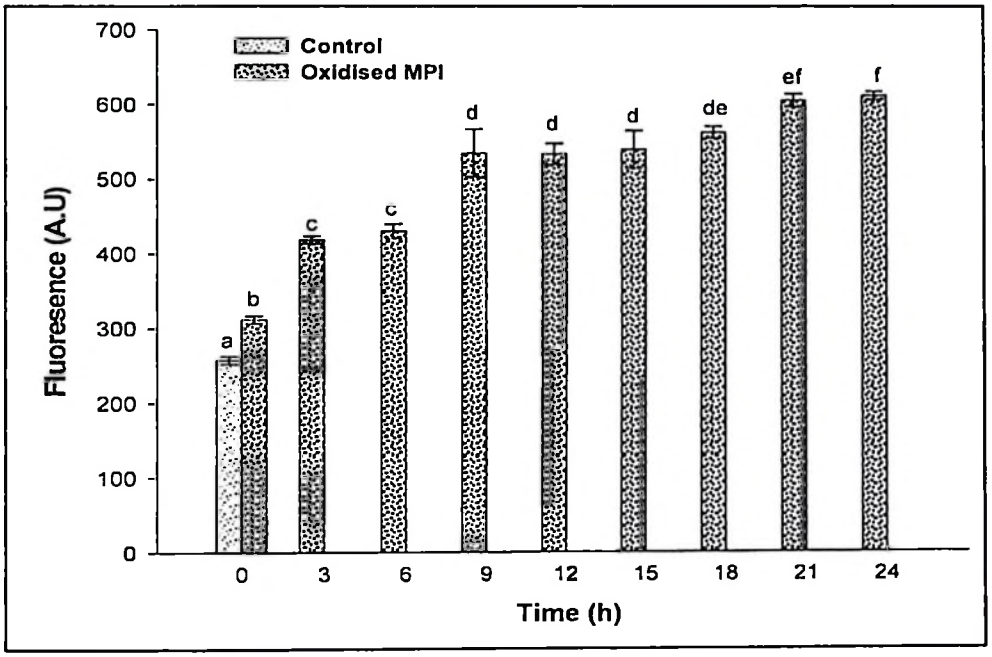


Figure 3.3 -Effect of different iron oxidation time on dityrosine formation of fish myofibrillar proteins. Dityrosine formation of fresh myofibrils is included as control. Groups with different letters (a, b, c, e, f) are significantly different (*P<0.05*).

3.1.6 SDS Gel Electrophoresis

SDS-PAGE for MPI, was performed to monitor oxidation induced polymerization or fragmentation of the oxidized MPI. IOS altered the electrophoretic pattern of MPI. Compared to control (MPI) oxidized MPI in the presence of β-mercaptoethanol showed decreased band intensity of myosin heavy chain (MHC) (Figure 3.4A). Simultaneously, new peptide bands with lower molecular masses, mainly in the range of 66.2 - 200 kDa, appeared, indicating that oxidation caused fragmentation of myosin. This observation is clearly supported by GPC results (Table 2 &Figure 3.5). Also a number of studies have shown that reactive oxygen species generated from metal oxidation system can cause a

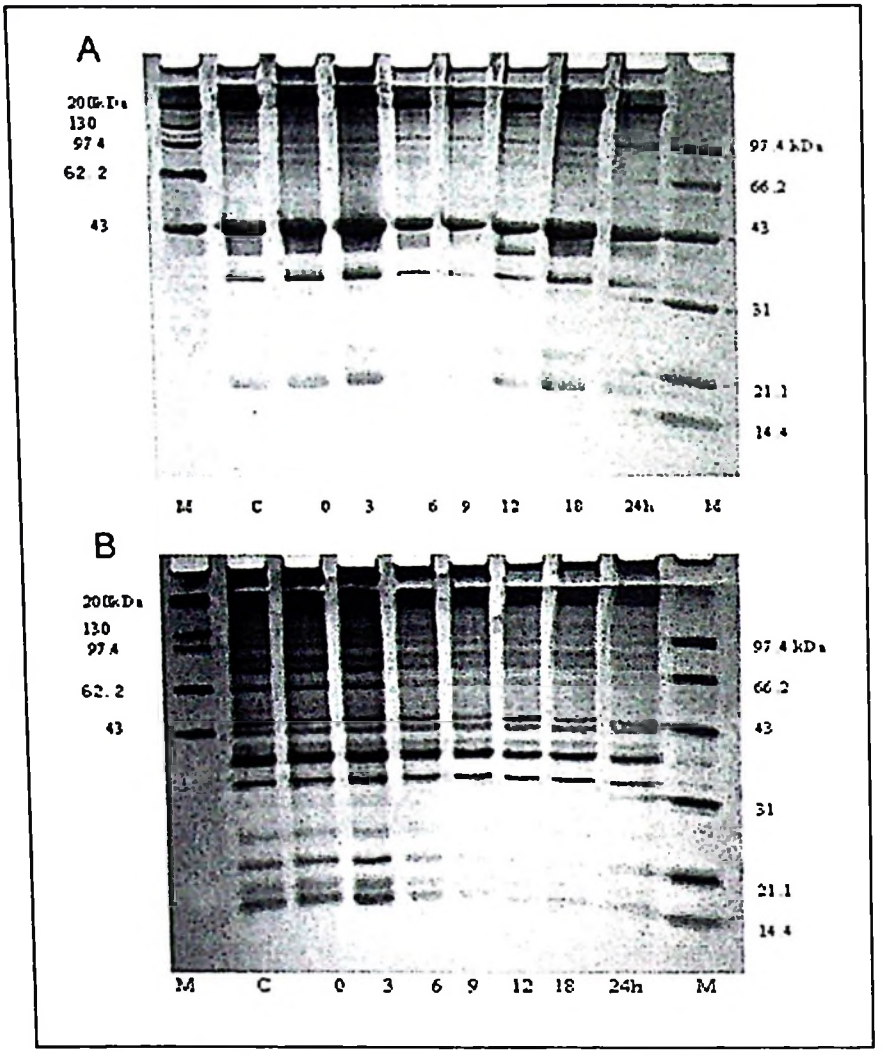


Figure 3.4 -Gel electrophoretic pattern of myofibrillar proteins after oxidation in the presence (a) and absence (b) of β- mercaptoethanol .Oxidation was performed at 4°C for 24h.Each lane was loaded with 20μg of protein.M- standard protein markers, C-Fresh myofibrillar protein.

wide variety of reactions on protein molecules, including modification of amino acids, fragmentation, and aggregation (Davies 1987; Morzel and 2006; Tokur and Korkmaz 2007b).Upon exposure of MPI to IOS resulted in a gradual and eventual decrease in intensity of actin and myosin which associated with production of polymers that barely entered the resolving gel irrespective of the increasing oxidation time (Figure 3.4B).It revealed that IOS caused dramatic changes in protein migration and a significant loss of actin and myosin (Figure 3.4 A & B). The lost proteins were mainly recovered when the

oxidized MPI samples were treated with β-mercaptoethanol, a reducing agent (Figure 3.4A). Polymers, formed during oxidation, partially dissociated into myosin and actin as demonstrated by the presence of large molecular weight bands at the top of the resolving gel in β-mercaptoethanol treated samples (including the non iron oxidized control) ,similar to that observed by Xiong and others(2009).

In the presence and absence β-mercaptoethanol, lower molecular weight bands decrease their intensity irrespective of oxidation time, beyond 18h of oxidation time, were significantly reduced (Figure 3.4 A&B) due to other covalent bonds apart from disulfide links, produced via cysteine oxidation, which were mainly responsible for myosin loss (Xiong and others 2009).

3.1.7 Gel-permeation chromatography

The calibration curve ($y = -0.6495x + 8.8768$, $R^2 = 0.9466$ whereby; y=logMw, x=Retention time) was established using various protein standards with their respective retention time (660 kDa, 8.82 min; 200 kDa, 10.43 min;150 kDa, 10.61 min; 66 kDa, 10.96min; 29 kDa, 11.65min; 12 kDa, 11.97 min; 6.5 kDa, 12.489min; 1 kDa, 13.19min) and was used in the determination of the molecular weight of the unknown oxidized MPI, since the conditions for the separation of the sample and the standards were selected and used in an identical manner. A determined relationship between the molecular weight and elution volume (relative peak area) was used basing on relatively similar conditions of the standards and oxidized MPI. The molecular weights of the oxidized MPI determined in this way are relative values and are described as standard 'equivalent molecular weights'. This means that dependent on the structural and chemical differences between the sample and the standards, the molecular weights can deviate from the absolute values to a greater or a lesser degree. Table 2 & Figure 3.5 show that IOS induced cross linking of silver carp MPI with respect to oxidation time. IOS induced cross linking of MPI, resulting into very large aggregates (with retention time less than 7min) which were excluded by column (due to column limit). It clearly shows a progressive decrease of the native proteins (with retention time between 7 and 9 min) relative molecular weights ,however sharp decrease of native protein was observed from 3 to 9 oxidation hour, then it slightly increased to 12 oxidation hour and, finally almost decrease continuously to 24 oxidation hour. It also should be noted that that SDS gel electrophoresis shows clearly the decrease of the native protein to form IOS induced

aggregates (Figure 3.4A & B). Hand in hand peak area with a retention time greater than 9min, their relative molecular weights increased progressively, in almost the same trends followed by the decrease of native fish muscle protein, suggesting IOS caused aggregation and degradation of silver carp MPI.

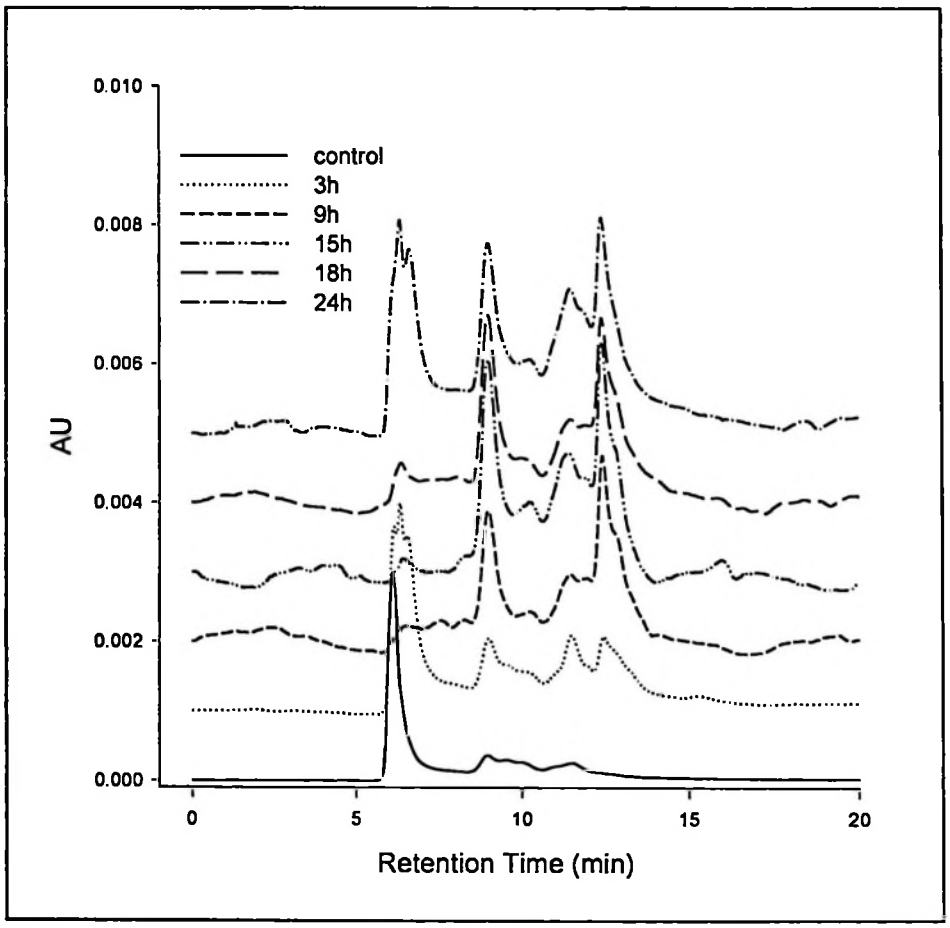


Figure 3.5 -Gel Permeation Chromatographic retention time of silver carp myofibrillar proteins subjected into iron oxidation system. The GP chromatography was carried out at elution rate 1mL/min with phosphate buffer solution (0.05M PBS) in 0.5 M NaCl (pH7.0) as an eluent.

Table2 – Gel Permeation Chromatographic relative peak area of oxidized silver carp MPI[1]

Oxidation time (h)	Percent peak area							
	P eak-1	P eak-2	P eak-3	P eak-4	P eak-5	P eak-6	P eak-7	P eak-8
0 (control)	63.16	9.55	6.81	5.09	15.38	ND*	ND*	ND*
0	1.70	65.27	0.31	1.25	3.84	8.61	19.02	ND*
3	1.22	68.36	7.54	1.97	20.92	1.22	ND*	ND*
6	0.48	7.62	64.40	13.08	14.42	ND*	ND*	ND*
9	0.53	16.09	1.59	48.42	4.07	1.11	28.18	ND*
12	0.24	6.32	1.16	5.40	56.68	ND*	3.52	14.73
15	1.73	43.24	11.45	43.59	ND*	ND*	ND*	ND*
18	4.52	45.95	5.92	38.12	4.52	ND*	ND*	ND*
21	19.64	19.32	21.33	19.44	20.27	ND*	ND*	ND*
24	19.40	16.69	17.11	20.61	23.92	ND*	ND*	ND*

[1]A Shodex- KW804 GPC Column with a protein exclusion limit of about 200 kDa was used for GPC. The GP chromatography was carried out at elution rate1mL/min with phosphate buffer solution (0.05M PBS) in 0.5 M NaCl (pH7.0) as an eluent. ND* not detected

3.1.8 The effect of iron-catalyzed oxidation system on protein functionality

Table 3 – Shows the PS, WHC, EC, OHC, and GS of myofibrillar proteins oxidized by IOS. Solubility of control (MPI without iron catalyst) in (0.5M NaCl) buffer was 92.12 %, compared to MPI subjected to IOS, it decreased slightly with the lapse of oxidation time and decreased to about 84.52% at the end of 24h iron catalyzed oxidation period. From the present study the decreased solubility in MPI suggest IOS induced aggregation of protein as supported by GPC results (Table 2 & Figure 3.5). In addition, protein carbonyl content and total –SH (Figure 3.1&3.2) demonstrated an increase of oxidation degree as a function of time causing aggregation of MPI which had negative impact on PS. This phenomenon is not good in muscle food since MPI solubility has been observed to be a prerequisite for many functional properties to attain desirable quality characteristics (Table 4).

Emulsification is one of the most important functionality in the manufacturing of many formulated foods. Emulsion represents a heterogeneous mixture of fat globulars. Food emulsion can be of the oil in water (O/W) or water in oil (W/O) type. The EC of MPI decreased significantly from 28.22mL/g at 0h oxidation to 25.21mL/g at 24h oxidation, suggesting that oxidation resulted into aggregate which was partially soluble, lowering protein adsorption at the interface. The decreased EC of the oxidized myofibrils was further manifested by significant reductions in the OHC of gels 0.62 mL/0.5g at 0h oxidation into 0.32 mL/0.5g at 24h oxidation.

WHC can be defined as the ability of a protein gel to retain water against a gravitational force. It reflects the extent of denaturation of the protein. Water content in this study was 6.18mL/g MPI at the beginning of oxidation time for sample subjected into IOS. It decreased with the lapse of oxidation time to 4.77mL/g at the end of 24h oxidation.

GS of the MPI was evaluated by the penetration force (Table 3), showed that GS of the control (without iron catalyst) was much higher than that subjected into IOS. This result indicated a continually decrease in the GS as a function of oxidation time, similar to that reported by Decker and others (1993). However, others reported increased gelling ability, water-holding capacity, and solubility under mild oxidizing conditions (Xiong and others 1993; Wan and others 1993). In this study, there was obvious decrease in GS as function of oxidation time.

Table 3 – The effects of an IOS on fish MP functionality in muscle food [1]

Oxidation time (h)	Silver MPI Functionality				
	SP (%)	EC (mL/g)	WHC (mL/g)	OHC (mL/g)	Gs(gf)
Control	92.16±2.237^{d}	26.73±0.71^{abcd}	6.17±0.37^{b}	0.55± 0.02^{abc}	98.22±2.35^{f}
0	91.11±1.70^{cd}	28.22±0. 42^{abcd}	6.58±0.33^{b}	0.61±0.01^{abc}	85.97±9.41^{e}
3	88.82±1.21^{abcd}	27.82±0 .10^{cd}	6.21±0.22^{b}	0.41±0.02^{abc}	70.51±6.71^{d}
6	89.14±1.56^{bcd}	27.42±0.23^{bcd}	6.03±0.27^{b}	0.60±0.13^{abc}	56.90± 2.30 ^{c}
9	88.92±0.80^{abcd}	27.42± 0.10^{bcd}	6.23±0.25^{b}	0.34±0.04^{ab}	51.84±1.03b^{c}
12	87.94±1.15^{bcd}	27.35±0.10^{bcd}	6.36±0.45^{b}	0.26±0.01^{a}	47.49±1.11b^{c}
15	86.28±1.49^{ab}	27.22±0.14^{bcd}	6.02±0.17^{b}	0.90±0.02^{c}	44.74±1.23^{b}
18	85.36±1.16^{ab}	26.23±0.29^{ab}	5.23±0.09^{a}	0 .72±0.54^{abc}	12.78± 0.24^{a}
21	86.79±0.96^{abc}	26.51±0.33^{abc}	5.22± 0.20^{a}	0.79±0.02^{bc}	12.34±0.21^{a}
24	84.52±2.33^{a}	25.21±0.11^{a}	4.77±0 .09^{a}	0.32±0.03^{ab}	12.08±1.13^{a}

1Means (3 replications, n = 3) in the same column without a common letter differ significantly ($P < 0.05$).

3.1.9 The correlation coefficient between oxidation parameters and Silver carp MPI functionality.

Results shown in Table 4 demonstrate significant *(P < 0.01)* and negative correlation between carbonyl content and PS (r=0.85) as well as GS (r=0.95), also dityrosine shows significant *(P < 0.01)* and negative correlation with PS (r=0.80) and gel strength (GS) (r= 0.95). On the other hand, the decrease in total thiol group content was significantly and positively correlated with PS (r=0.77) and GS (r= 0.89).The existence of these associations logically explains the alterations of MPI functional properties when exposed in an oxidative environment, to a great extent being influenced by the degree of oxidation. However, functional properties in addition to being influenced by the degree of oxidation as demonstrated by change in carbonyl content, total –SH and dityrosine formation are also seem to be influenced by their interaction with one another particularly solubility with other functional properties, for example gel strength.

Table 4 – Correlation coefficient between oxidation parameters and Silver carp MPI functionality[1]

Parameters	PS	EC	WHC	OHC	GS
Carbonyl content	-0.85*	-0.60**	-0.75**	0. 09	-0.95**
Total -SH	0.77**	0.44*	0.64**	0.02	0.89**
Dityrosine	-0.80**	-0.47**	-0.61**	0.03	-0.93**
PS	1.00	0.60**	0.72**	-0.04	0.85**
EC	0.60**	1.00	0.83**	0.04	0.60**

** [1]Pearson correlation coefficient (r) is significant at ($P < 0.01$), (2-tailed).

* [1]Pearson correlation coefficient (r) is significant at ($P < 0.05$), (2-tailed).

3.1.10 summary

The present investigation reveals that, changes in the functional properties of silver carp MPI were greatly influenced by loss of MPI solubility, which reflect, the changes in the native protein structure to a greater extent owing to impact of iron-catalyzed oxidation system. In addition, the results demonstrated that iron-catalyzed oxidation system has impact on functional changes of processed white muscle fish. Moreover, the results clearly show that the reduced protein functionality can be related to the changes in the chemical and physical properties of oxidized proteins as confirmed by SDS-PAGE and GPC results. Therefore, to maximize the

functionality of muscle proteins during silver carp processing and to enhance the utilization of it as a low value fish that is susceptible to oxidation, it is necessary to design processing procedures and formulations that would minimize protein oxidation by incorporating antioxidants.

3.2 The influence of fatty acids oxidation on protein functionality for frozen stored silver carp myofibrillar protein

3.2.1 Protein carbonyl content

The protein carbonyl content of frozen stored myofibrillar protein with 0.6M NaCl or isolated from previous frozen stored whole silver carp and fillets are shown in Figure 3.6. MPI frozen stored with 0.6 M NaCl and those from frozen stored whole fish and fillet presented a similar trend in relation to MPI stored with 0.1M NaCl (control).However, MPI isolated from frozen stored whole fish showed a high degree of protein oxidation. This is due to different level of fat in whole fish/fillet (1.2%) and MPI (0.6%).There are two main reasons contributed to the observed differences in fat content in this study. Firstly, myofibrillar protein was isolated from dorsal part of the fish which has relatively low fat content and secondly, isolation involved washing of the fillet with sample buffer, also this contributed significantly to the variation. The interaction of polyunsaturated fat acid and protein in the storage system is assumed to be the source and contributed to observed difference in oxidation degree between treatments. Consistent with the suggestion made by Xiong and Decker, (1995) that free radicals generated via lipid oxidation were involved in the oxidation of myofibrillar protein since the myofibril samples contained a residual amount of lipids (0.5%),which is less than 0.6% residual lipid in silver carp MPI.

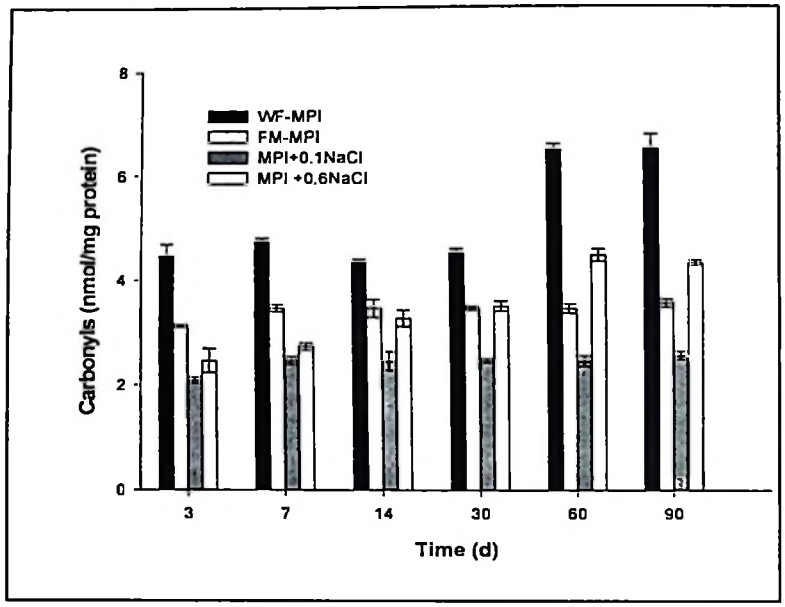


Figure 3.6 – Formation of protein carbonyls MPI of silver carp stored at -18°C for 90 days. Legends described in method section.

3.2.2 The change in total sulfhydryls content of frozen silver carp MPI

The data in Figure 3.7 present a similar trend throughout the storage period; it can be observed that myofibrillar isolated from frozen whole fish always presented higher degree of protein oxidation than other treatments. Through the end of frozen storage, it is also observed that total –SH is generally lower in MPI stored with 0.6M NaCl than other treatments and control(MPI with 0.1M NaCl).It is possible that sodium chloride had no influence in controlling the level of protein oxidation by lowering water activity. Therefore, observed change in total sulfhydryl content of frozen silver carp MPI mainly is due conformational changes, taking place in proteins during frozen storage, leading to a continuous exposure and burial of -SH groups. SH groups are prone to oxidation but are also involved in interchange reactions as well as in metallic complexes (Stadtman and Levine 2003; Buttkus, 1971).

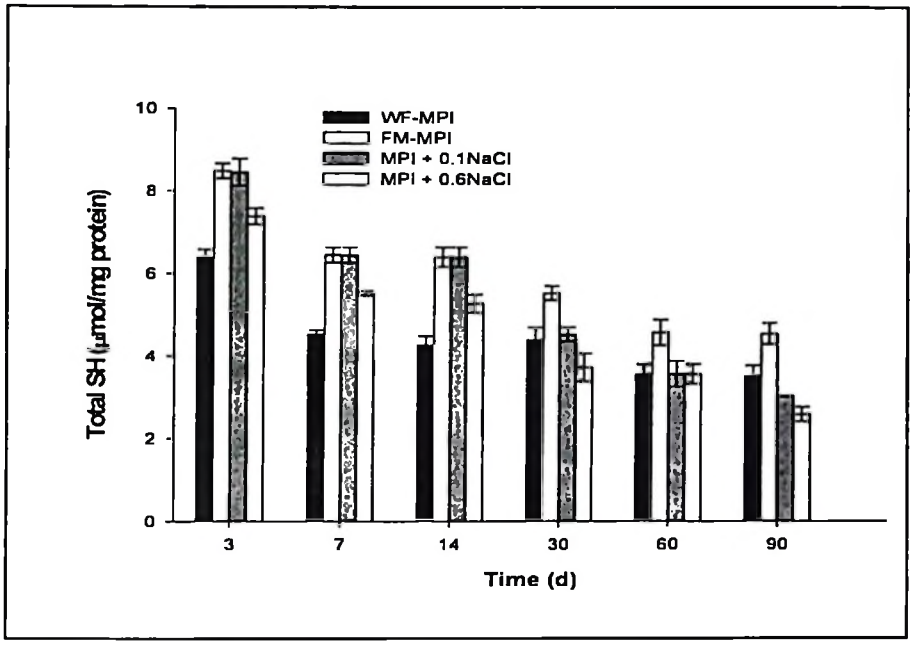


Figure 3.7 – Changes of SH groups in silver carp MPI stored at -18°C for 90 days. Legends described in method section.

3.2.3 Determination of dityrosine formation

Results of dityrosine test are plotted in Figure 3.8 the variability of results prevents to arrive to an immediate conclusion. Nevertheless initial values were lower for myofibrillar isolated from frozen fish mince than for whole fish. This may be due to the inferior fat content in minced silver carp compared to whole one with extra fat in the skin and ventral part.

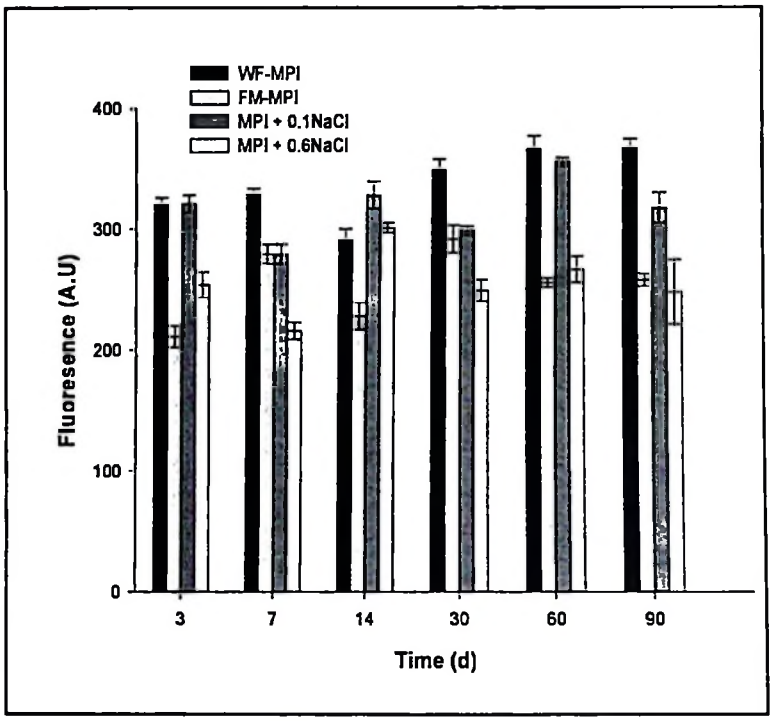


Figure 3.8 – Changes of dityrosine formation in silver carp MPI stored at -18°C for 90 days measured by fluorescence. Legends described in method section.

3.2.4 Differential scanning calorimetry

DSC endothermic transitions temperatures of silver carp MPI stored with sodium chloride, both fresh and frozen stored WF-MPI and FM-MPI are shown in Table 5. The transition temperatures of myosin for sample MPI stored with 0.1M NaCl and 0.6M NaCl were 48.12°C and 46.40°C, respectively. This suggests high ionic strength contributed by sodium chloride increased susceptibility of myosin to thermal denaturation.

Table 5 – The onset (Tonset) and maximum (Tmax) temperatures for endothermic transitions and net heat energy (enthalpy, ΔH) required for these transitions for silver carp MPI stored at -18°C for 90 days

Treatment code	Peak 1			Peak 2			Peak 3		
	T_{onset} (°C)	T_{max} (°C)	ΔH (J/g)	T_{onset} (°C)	T_{max} (°C)	ΔH (J/g)	T_{onset} (°C)	T_{max} (°C)	ΔH (J/g)
F-MPI[1]	45.12	69.80	119.20	76.42	79.06	8.60	80.79	84.89	26.60
0.1NaCl- MPI	43.29	48.12	4.53	ND*	ND*	ND*	ND*	ND*	ND*
0.6NaCl- MPI	44.11	46.40	0.60	55.73	59.74	2.91	ND*	ND*	ND*
WF-MPI	42.04	45.26	1.49	ND*	ND*	ND*	ND*	ND*	ND*
FM-MPI	35.17	48.05	13.84	56.53	58.78	0.32	60.67	82.60	125.86

F-MPI[1] is a Fresh myofibrillar isolated within 24 of DSC measurement. ND*-not determined, Treatment code is defined in method

3.2.5 The peroxide value (POV)

The peroxide value results are presented in Figure 3.9. In the first 7days of frozen storage for both myofibrillar isolated from fish mince and whole fish showed a sharp increase in POV of silver carp stored at -18°C. The peroxide value of frozen MPI is a useful indicator of decomposition during the early stages of oxidation, but is of lesser value once the oxidation resulted into formation of secondary lipid oxidation. The observed decline in POV for myofibrillar isolated from fish mince for 14 days frozen storage suggest that POV measurement is not reliable in assessing the oxidation of MPI composed of polyunsaturated fatty acid (Table 1).POV likely formed unstable peroxides which react quickly to form secondary products. Thus in our study POV has been used in conjunction with TBA test.

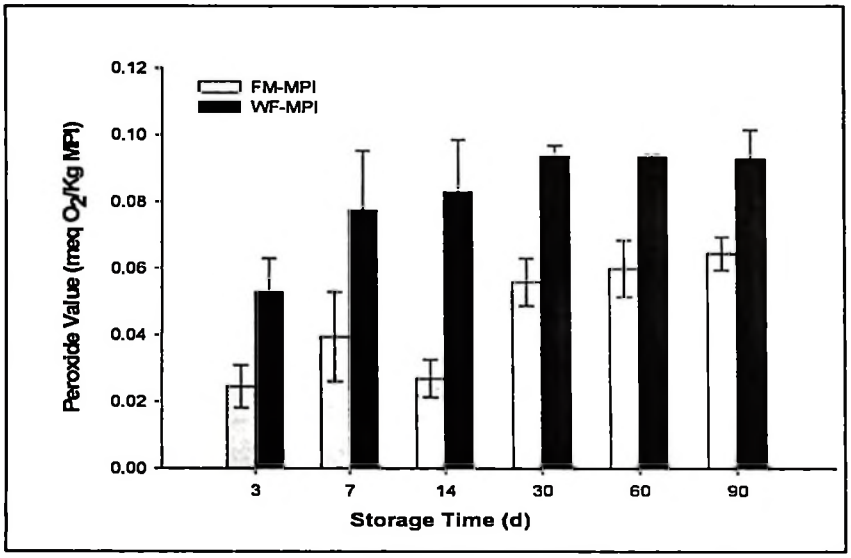


Figure 3.9 Peroxide values obtained from silver carp MPI stored at -18°C. Legends described in method section.

3.2.6 The TBARS test

Results of TBARS test are plotted in Figure 3.10.The TBARS test was also used to monitor the oxidation for up to 90days.There was an increase in TBARS in both myofibrillar isolated from whole fish and fish mince frozen stored. There were also significant different observed between the levels of TBARS from 3 to 30 days of the two MPI sources .This implies fish mince is a better form for frozen storage as raw materials for myofibrillar isolation .As this study was undertaken on fish MPI does not agree with several published results on the difference reported on whole fish and fish fillet/mince on susceptibility to oxidation on frozen storage. They reported fish fillet/ mince are more susceptible to oxidation compared to whole fish (Saeed and

Howell 2002).This discrepancy is based on differences of fish species, in this study lean fish and mainly dorsal part was used as a source MPI. The observed difference in functionality for the different sources is supported by Smith, (1987), that muscle foods subjected to comminuting processes, such as mechanical deboning, show decreased protein functionality which could be due, in part, to the oxidation.

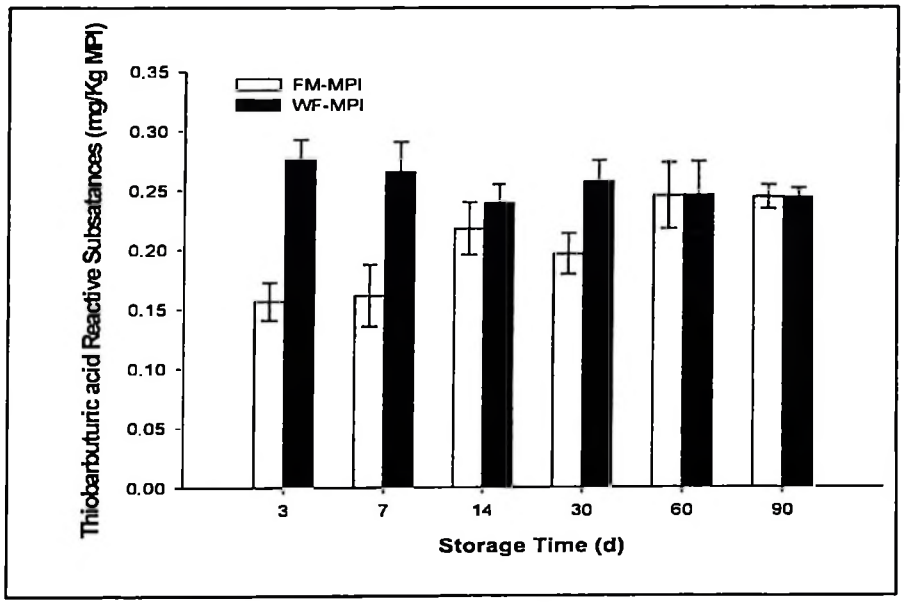


Figure 3.10 Thiobarbuturic acid reactive substances obtained from silver carp MPI stored at -18°C.

3.2.7 The change of total and free amino acid of silver carp WF, FM and MPI

As seen in Table 6, there was an obvious difference between whole fish and/or fish mince and myofibrillar protein isolate with respect total amino acids after 90days of frozen storage. There was a great loss of amino acids such as cysteine, lysine, histidine and methionine, for whole fish frozen stored at -18°C compared to fish mince and myofibrillar protein isolate. These observations imply, whole fish was more susceptible to oxidation compared to fish mince and myofibrillar protein isolate. In part due to their relatively high level of lipid in their skin, this observation agrees well with that reported by Saeed and Howell (2002) that alteration myofibrillar protein of many fish species were caused by their interaction with different type of lipids or lipids oxidation products during frozen storage. However, these results do not agree with that reported by Xiong and Decker (1995) that oxidation is facilitated by processes such as comminuting and grinding, which introduce molecular oxygen and mix with oxidants making meat components susceptible to oxidation. For further comparison of these three forms of frozen storage their free amino acid are shown in Table 7.

Table 6 – Total amino acid compositions of frozen stored silver carp for three months

Amino acid	Control[1]	MPI g/100g	FM g/100g	WF g/100g
Asp	27.47	11.37	11.21	11.36
Glu	20.22	18.84	18.90	20.65
Ser	2.22	4.73	4.75	4.18
His	0.77	1.96	2.12	1.85
Gly	30.20	3.97	4.37	4.31
Thr	0.68	4.48	4.62	4.025
Arg	1.54	6.42	6.51	7.53
Ala	1.19	5.38	5.81	5.51
Tyr	0.09	4.25	4.12	4.42
Cys	0.09	0.99	0.85	0.73
Val	0.26	4.41	4.41	4.10
Met	0.09	2.29	2.73	2.53
Phe	0.85	3.68	3.73	4.90
Ile	0.34	4.14	4.19	3.71
Leu	0.43	8.49	8.69	7.36
Lys	0.94	9.64	9.79	8.36
Pro	1.45	2.91	2.740	3.95
Trp	11.18	2.03	0.44	0.53
TOTAL	100	100	100	100

Control[1] – Amino acid of silver carp fresh fillet

Table 7 – Free amino acid compositions of frozen stored silver carp for three months

Amino acid	Control[1]	MPI g/100g	FM g/100g	WF g/100g
Asp	23.40	24.89	12.11	28.72
Glu	20.21	22.59	10.09	23.09
Ser	0.71	0.02	0.09	0.00
His	0.00	0.01	0.30	0.00
Gly	23.4	15.61	6.96	17.76
Thr	1.06	0.99	0.57	1.04
Arg	1.42	0.79	1.45	0.89
Ala	0.00	0.40	0.66	0.81
Tyr	0.00	0.01	1.63	0.59
Cys-s	2.82	3.23	0.24	1.11
Val	0.00	0.40	0.72	1.04
Met	0.00	0.33	0.15	0.52
Phe	0.00	0.46	1.36	0.59
Ile	0.00	1.25	0.21	2.07
Leu	2.13	0.00	1.24	0.30
Lys	0.00	1.05	0.24	1.70
Pro	1.06	4.68	59.62	12.36
Trp	25.18	23.31	2.38	7.40
TOTAL	100	100	100	100

Control[1]—Amino acid of silver carp fresh fillet

3.2.8 The solubility and water holding capacity of myofibrillar proteins

Table 8 — Shows the solubility and water holding capacity of myofibrillar proteins stored at -18°C for 90 days. Solubility of both WF-MPI and FM-MPI in (0.5MNaCl) buffer were 82.7 % and 82.2% respectively after 3 days of storage, they decreased significantly ($P<0.05$) with the extension of storage period and decreased to about 73.9% and 75.6%,respectively at the end of storage period. On the other hand solubility of MPI stored with 0.1NaCl decreased significantly ($P<0.05$) after 30days of frozen storage, whereas for that of MPI stored with 0.6 NaCl, its solubility was high compared to the former but did not differ significantly($P>0.05$) with respect to storage time. The decrease in PS may be attributed to the protein denaturation and protein aggregation induced by frozen storage (Grabowska and Sikorski, 1974).

On the other hand, oxidized lipids interact with amino acids in fish proteins, such as cysteine - SH, the -NH2 group of lysine, and the N-terminal groups of aspartic acid, tyrosine, methionine and arginine;

these interactions increase the hydrophobicity of proteins, thus increasing aggregation (Kussi and others 1975).

Water content in this study for all treatments differ significantly *(P<0.05)* from the beginning to the end of frozen storage. The changes in the water holding capacity for all treatments, frozen stored for 90 days at -18°C might have resulted from the freezing process and the length of time WF, FM and MPI remained in cold storage. However, it seems that the freezing process exerted a more profound effect on the water holding capacity of the frozen fillets stored for 90days than did the storage time. Moreover, the addition of sodium chloride to 0.6M at pH6.5 increased water holding capacity, this observation agrees with that reported by Kristin and others, (2002) that adding sodium chloride above the isoelectric points of the proteins in meat system causes swelling and an increase of water holding capacity.

Table 8 – Protein functionality of MPI and MP isolated from frozen stored whole fish and fish mince of Silver carp[1]

Storage time (d)	Solubility (%)				Water holding capacity (mL/g)			
	WF-MPI	FM-MPI	0.1NaCl	0.6NaCl	WF-MPI	FM-MPI	0.1NaCl	0.6NaCl
3	82.7± 2.52^{c}	82.8 ± 1.78^{b}	85.1 ± 0.41^{b}	87.4 ± 0.33^{a}	6.2± 0.32^{b}	6.0 ± 0.27^{b}	6.2 ± 0.32^{b}	7.6 ± 0.33^{b}
7	78.11±1.69^{c}	79.5±2.69^{ab}	82.6±0.17^{b}	87.5±0.21^{a}	6.2 ± 0.22^{b}	6.2 ± 0.24^{b}	6.2 ± 0.22^{b}	7.4 ± 0.37^{b}
14	76.49 ± 2.26^{b}	81.2 ± 1.86^{b}	81.7± 0.25^{b}	86.7± 0.24^{a}	6.0 ± 0.17^{b}	6.5 ± 0.31^{b}	6.2 ± 0.24^{b}	7.7 ± 0.26^{b}
30	75.85 ± 1.55^{b}	78.9 ± 1.17^{ab}	77.1 ± 1.50^{a}	82.1 ± 1.56^{a}	5.2 ± 0.20^{a}	6.0 ± 0.17^{a}	6.0 ±0.17^{b}	7.2 ± 0.09^{b}
60	75.9 ± 1.80^{b}	79.1 ± 1.56^{ab}	76.9 ± 0.80^{a}	81.9 ± 0.80^{a}	5.3 ± 0.15^{a}	5.2 ± 0.09^{a}	5.2 ±0.20^{a}	6.8± 0.09^{a}
90	73.9 ± 0.50^{a}	75.6 ± 0.58^{a}	76.0 ± 1.15^{a}	80.9 ± 1.15^{a}	5.4 ± 0.17^{a}	5.2 ± 0.20^{a}	5.3 ±0.15^{a}	6.4 ± 0.17^{a}

[1]Means (3 replications, n = 3) in the same column without a common letter differ significantly ($P < 0.05$).

Table 9 – Protein functionality of MPI and MP isolated from frozen stored whole fish and fish mince of Silver carp[1]

Storage time (d)	Emulsification capacity (mL/0.5g)				Oil holding capacity (mL/g)			
	WF-MPI	FM-MPI	0.1NaCl	0.6NaCl	WF-MPI	FM-MPI	0.1NaCl	0.6NaCl
3	23.0 ± 1.80^{b}	24.4 ± 1.80^{b}	23.1± 1.26^{b}	24.7± 1.26^{b}	0.5 ± 0.01^{c}	0.5 ± 0.02^{b}	0.6 ± 0.02^{b}	0.6 ±0.01^{a}
7	22.8 ± 1.15^{b}	23.7 ± 1.15^{b}	22.7 ± 0.76^{b}	24.3 ± 0.76^{b}	0.5 ±0.01^{c}	0.5 ±0.001^{b}	0.6±0.02^{b}	0.6±0.03^{a}
14	23.4 ± 1.18^{b}	24.7 ± 0.50^{b}	22.9 ± 0.60^{b}	24.1 ± 0.70^{b}	0.4 ± 0.02^{ab}	0.3 ± 0.02^{ab}	0.6 ±0.02^{b}	0.6 ± 0.02^{a}
30	21.4 ± 1.15^{a}	23.7 ± 1.15^{a}	21.4± 0.39^{a}	23.4 ± 0.39^{a}	0.4 ± 0.13^{b}	0.5 ± 0.03^{b}	0.5 ± 0.01^{a}	0.6 ± 0.01^{a}
60	22.0 ± 1.42^{a}	23.5 ± 0.81^{a}	21.4 ± 0.39^{a}	23.4 ± 0.18^{a}	0.3 ± 0.04^{a}	0.3 ± 0.04^{a}	0.5 ± 0.03^{a}	0.6 ± 0.03^{a}
90	21.4 ± 0.81^{a}	23.1± 1.71^{a}	22.00 ±1.50^{a}	24.0 ±1.00^{a}	0.2 ± 0.010^{a}	0.2 ± 0.01^{a}	0.5 ± 0.02^{a}	0.6 ± 0.02^{a}

[1]Means (3 replications, n = 3) in the same column without a common letter differ significantly ($P < 0.05$).

3.2.9 The emulsifying capacity and oil holding capacity of myofibrillar proteins

There was a significant ($P<0.05$) difference with respect to EC values in MP isolated from frozen stored whole fish and fish mince and MPI stored with sodium chloride for 90 days at -18°C (Table 9). Normally two factors affect the EC of protein and these are the amount of soluble protein available and the efficiency of the protein to emulsify fat (Saffle, 1960). The decline in EC values observed in the present study is attributed to the decrease in muscle protein solubility. OHC of MPI for most of the treatments did not differ significantly ($P>0.05$) in the entire frozen storage period. This observation suggests that storage time did affect OHC as shown in Table 9.

3.2.9 Summary

The addition of sodium chloride to 0.6M at pH6.5 improved protein functionality especially water holding capacity of frozen stored myofibrillar protein isolate. However, the differential scanning calorimeter results showed that, sodium chloride significantly increased thermal susceptibility of myosin.

Whole fish frozen storage was more susceptible to oxidation compared to fish mince and myofibrillar protein isolate due to their different lipid content. Also myofibrillar isolated from frozen whole fish showed significant changes in protein functionality may be due to great loss of amino acid such as cysteine, lysine, histidine and methionine during frozen storage. It can, therefore, be concluded that frozen storage of silver carp fish mince and myofibrillar protein isolate at -18°C caused a small but significant decrease in the functionality. Moreover, the results clearly show that the reduced protein functionality can be related to the changes in the chemical and physical properties of oxidized proteins as confirmed by DSC results.

3.3 The effects of cryoprotectants and antioxidants on the functional properties of frozen myofibrillar protein isolate

3.3 .1 Protein carbonyl content

The protein carbonyl content significantly remain unchanged in the control after 90 days of frozen storage, but for the MPI treated with cryoprotectants there was significant increase in carbonyl content in the entire storage time compared to the control. On the other hand, in the first 30 d and 90 d of frozen storage there were a significant difference between the samples stored with cryoprotectants and antioxidants, unexpectedly after 60 d of frozen storage there was no significant difference between control and samples stored with cryoprotectants and antioxidants (Figure 3.11). This odd observation is assumed to be due to the interaction effects of MPI, cryoprotectants and antioxidants based on observed increase of carbonyl contents for all samples stored with 8% cryoprotectants. In addition the low degree of protein oxidation observed was mainly contributed by low storage temperature (-18°C) and antioxidants. In combination of 8% cryoprotectants and (0.2%) ascorbate (MPI+S+A), ascorbate was found to be effective in controlling the oxidation of MPI stored at -18°C in the first 30 d of storage, however ,it imposed a negative effect by increasing carbonyl content in the last 60 d of frozen storage. Similarly (Srinivasan and Xiong 1996), observed change in carbonyl content (protein oxidation) for sample stored with ascorbate during the storage of beef heart surimi- like materials. Essentially the carbonyl content of proteins remained unchanged during storage in the presence of 8% cryoprotectants and 0.2% α-tocopherol (MPI+S+E) in the whole storage period, despite the similarity with control observed in first month of storage. The α-tocopherol alone was found to be the most effective in controlling oxidation of MPI stored at -18°C for 90d (Figure 3.11). On the other hand the combination of 8% cryoprotectants 0.1%ascorbate and 0.1% α-tocopherol (MPI+S+A+E) reduced oxidation ,mean while these combination in the first 30 d of storage was found to behave in similar fashion as when ascorbate when was used alone.

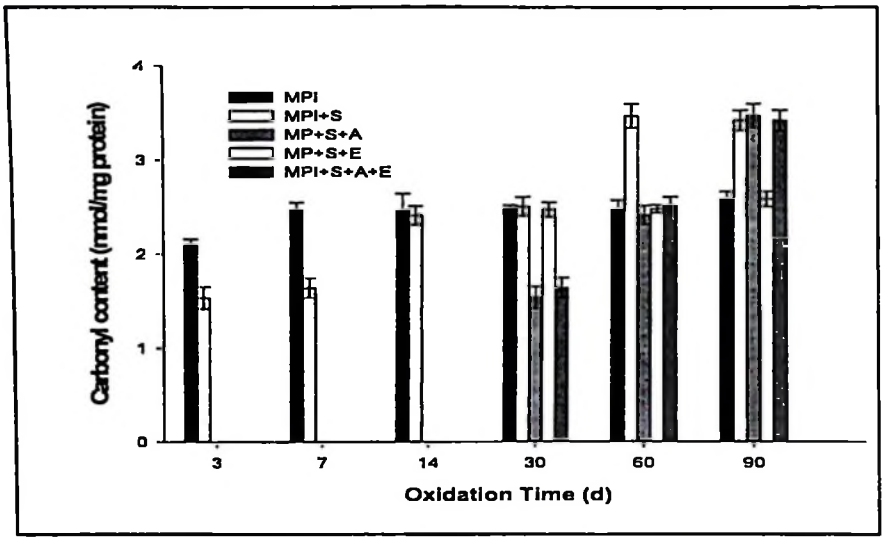


Figure 3.11 -Formation of protein carbonyls in silver carp stored at -18°C for 90 days. Legends described in method section.

3.3.2 The total sulfhydryls content of silver carp MPI

The total number of -SH groups diminished significantly in the control during 90 days of frozen storage, with regard to it, MPI stored with either cryoprotectants or mixture of cryoprotectants and antioxidants also decrease at relatively low rate in the entire storage period (Figure 3.12). The addition of the mixture of sucrose and sorbitol reduced such decreases notably at the end of 90 days storage. However, no differences were found between cryoprotectants and control in the first 60 d of storage.

Conformational changes, taking place in proteins during frozen storage, lead to a continuous exposure and burial of -SH groups. -SH groups are prone to oxidation but are also involved in interchange reactions as well as in metallic complexes (Stadtman and Levine 2003; Buttkus, 1971). Basing on that, samples stored with cryoprotectants or mixture of cryoprotectants and antioxidants, prevented a rapid exposure of sulfhydryls groups on the protein surface, which in turn slowed the loss of SH groups. Meanwhile, unfolding and aggregation took place rapidly in the control, i.e. during 90 days of frozen storage, resulting into a significant decrease of SH groups.

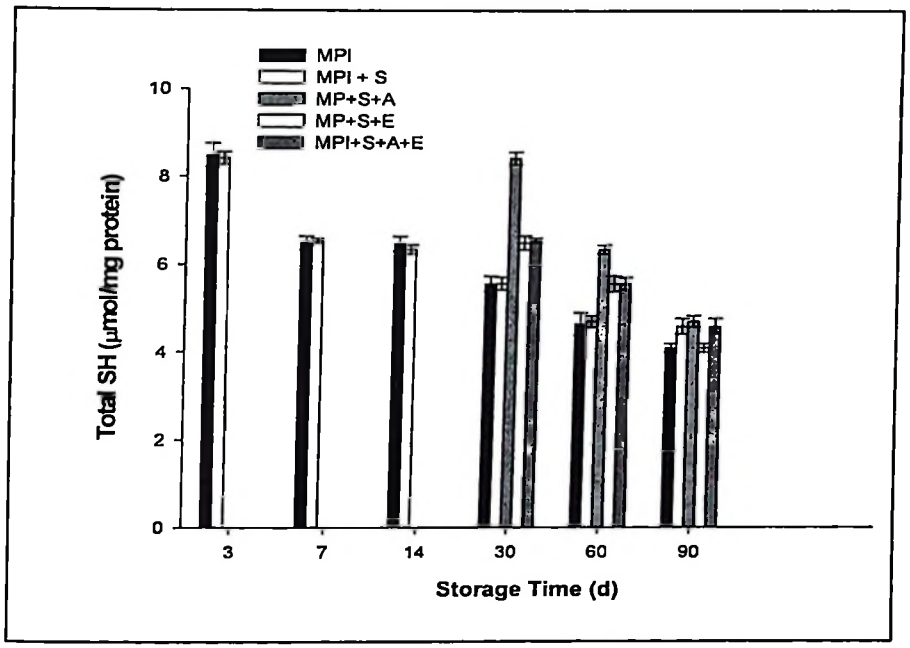


Figure 3.12- Changes of SH groups in silver carp MPI stored at -18°C for 90days. Legends described in method section.

3.3.3 Determination of dityrosine formation

Oxidative damage, particular to proteins, has been widely postulated to be a major causative factor in the loss of functional properties during storage. Tyrosine oxidation products can be used as markers for oxidative stress invitro due to the formation of dityrosine upon oxidation which has distinct fluorescent properties as described to be a useful indicator for protein modification (Davies 1987). Thus in this study, dityrosine formations decreased significantly for MPI stored with either cryoprotectants or mixture of cryoprotectants and antioxidants. This observation suggests that, antioxidants protected protein from oxidation during storage (Figure 3.13).

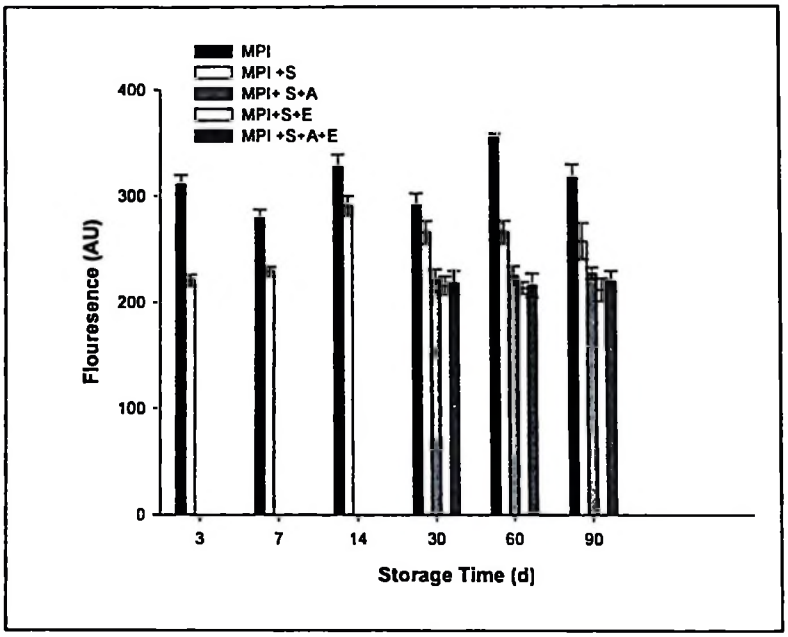


Figure 3.13 -Changes of dityrosine formation in silver carp MPI stored at -18°C for 90days measured by fluorescence. Legends described in method section.

3.3.4 SDS Gel Electrophoresis

SDS-PAGE for MPI stored at -18°C, was performed to monitor the storage induced polymerization or fragmentation of frozen MPI. Electrophoresis indicated that almost native myofibrillar protein of silver carp either with or without antioxidant remained intact in the first 60 days of frozen storage (Figure 3.14 A&B). In the absence of β-mercaptoethanol (Figure 3.14B) there was a considerable formation of new peptide bands with lower molecular masses, about 97 kDa, indicating that frozen storage caused fragmentation of myosin. As storage time increased at -18°C, both myosin and actin bands were still strong visible after 90 days of frozen storage (Figure 3.14C&D) for MPI stored with antioxidants, however the myosin heavy chain (MHC) disappeared in samples stored without antioxidants. Therefore using antioxidants prevented loss of myosin indicating protection of myosin from lipid oxidation products and from aggregation in frozen storage. This observation is in agreement with that reported by (Saeed and Howell 2002) on the study of frozen storage of Atlantic mackerel.

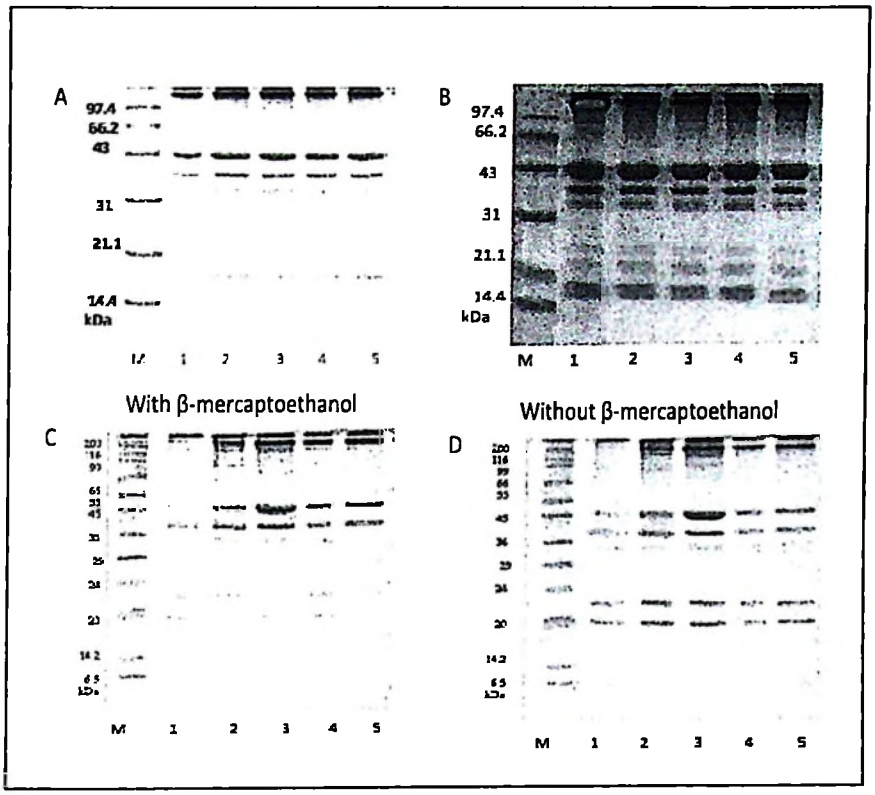


Figure 3.14 -SDS-PAGE separation of MPI silver carp stored at -18°C for 60 and 90 days A&B and C&D respectively without cryoprotectants and antioxidants (lane 1) or with cryoprotectants (lane 2) or antioxidants 0.2% ascorbate(lane 3),0.2% α-tocopherol (lane 4) or 0.1% ascorbate + 0.1% α-tocopherol(lane 5). M- Marker range 14.4 -97.4kDa is shown in lane M.

3.3 .5 Differential scanning calorimetry (DSC)

DSC is a useful technique for studying thermal behavior of muscle proteins as a means of gaining information about protein folding and stability in biochemical systems (Wright and others 1977). It is a useful technique in studying thermally induced transitions in fish proteins as a function of species and processing condition (Hastings and others, 1985; Park and Lanier, 1989). Changes in protein structure during DSC analysis are referred to as "transition" changes, and peak temperatures at these transitions are used to represent transition temperatures (Jittinandana and others, 2003). A lower transition temperature indicates that the protein is more susceptible to thermal denaturation (Jittinandana and others, 2003). Different fish species possess different transition temperatures for myofibrillar proteins (Hastings and others, 1985).

DSC endothermic transitions temperatures of silver carp MPI, both fresh and frozen stored without cryoprotectants or with cryoprotectants or mixture of cryoprotectants and

antioxidants are shown in Table 10. The transition temperatures of myosin and actin in fresh silver carp MPI were 45.12°Cand 76.42°C, respectively. The endothermic transition with a peak temperature at 66.80°C, may be associated with denaturation of the myosin and the second, at 78.24°C, was due to that of the actin, both being contractile myofibrillar proteins. These results agree with those reported by Samejima and others (1981) that myosin head (S-1 fraction) has a lower temperature of denaturation (43 °C) than the rod fraction (55 °C) determined by DSC.

Myosin is susceptible to thermal denaturation, frozen storage increased myosin susceptibility to thermal denaturation by lowering the transition temperature from 45.12°C to 43.29°C. The endothermic transition with a peak temperature at 66.80°C for myosin was lowered to 48.12°C.On the other hand, cryoprotectants increased myosin transition temperature from 43.28°C to 47.85°C for the first peak and to 56.09°C for the second peak compared to MPI frozen stored without cryoprotectants. This observation is clearly supported by SDS-PAGE results (Figure 3.14C&D). MPI stored without cryoprotectants did not show any transition temperature for actin, cryoprotectants reduced actin susceptibility to thermal denaturation during storage, however compared to fresh silver carp MPI the endothermic transition with a peak temperature at 78.24°C was lowered to 66.80°C for frozen MPI with cryoprotectants at -18°C for 90 days. The combination of cryoprotectants and antioxidants did not significantly protect silver carp MPI against thermal denaturation during frozen storage (Table 10), thus cryoprotectants proved to protect well myofibrillar protein against thermal denaturation mainly when used without antioxidants. This suggests that interaction effects of cryoprotectants and antioxidants were not in favor of myofibrillar protein against thermal denaturation.

Table 10 – The onset (T_{onset}) and maximum (T_{max}) temperatures for endothermic transitions and net heat energy (enthalpy, ΔH) required for these transitions for silver carp MPI stored at -18°C for 90 days

Treatment code	Peak 1			Peak 2			Peak 3		
	T_{onset} °C)	T_{max} (°C)	ΔH (J/g)	T_{onset} (°C)	T_{max} (°C)	ΔH (J/g)	T_{onset} (°C)	T_{max} (°C)	ΔH (J/g)
F-MPI[1]	45.12	69.80	119.20	76.42	79.06	8.60	80.79	84.89	26.60
0.1NaCl- MPI	43.29	48.12	4.53	ND*	ND*	ND*	ND*	ND*	ND*
MPI+S	44.85	56.09	2.62	ND*	ND*	ND*	65.00	72.00	1.67
MPI +S+A	37.27	38.58	0.02	54.99	59.99	-89.69	ND*	ND*	ND*
MPI+S +E	47.37	51.94	4.32	ND*	ND*	ND*	72.89	76.44	4.30
MPI+S+A+E	46.00	47.59	0.04	48.81	50.09	0.03	ND*	ND*	ND*

Fresh MPI[1] =Myofibrillar protein isolated within 24h of DSC measurement, ND* not determined, Treatment code is defined in method section

3.3 .6 The effect of cryoprotectants and antioxidants on frozen silver carp MPI protein functionality

GS of silver carp MPI stored for 90 days was evaluated by the penetration force, Figure 3.15- show that GS of the control (without cryoprotectants or cryoprotectants and antioxidants) decreased with respect of storage time. This result implies, oxidation was taking place in frozen storage probably at a slow rate causing a continually decrease in the GS as a function of storage time, similar to that reported by Decker and others (1993). In this study cryoprotectants or cryoprotectants and antioxidants improved GS of silver carp MPI in the entire storage period (Figure 3.15).

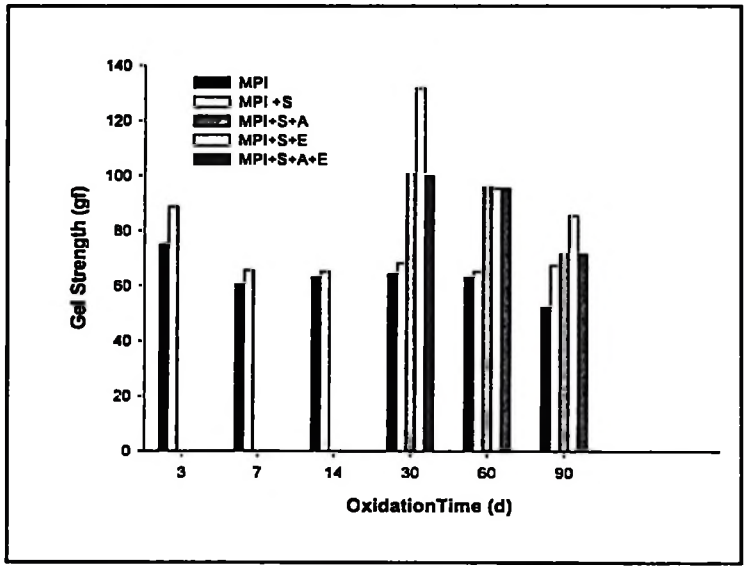


Figure 3.15- The effect of cryoprotectants and antioxidants on GS of silver carp MPI stored at -18°C for 90days. Legends described in method section.

Table 11- Shows the solubility and water holding capacity of myofibrillar proteins stored at -18°C for 90 days. Solubility of the control (MPI without cryoprotectants or cryoprotectants and antioxidants) in (0.5M NaCl) buffer was 77.9 %, at the first 30 days of storage, it decreased significantly ($P<0.05$) with the extension of storage period and decreased to about 74.6% at the end of storage period. From the present study the cryoprotectants or cryoprotectants and antioxidants improved solubility of MPI in the whole storage period suggesting that they protected MPI against denaturation and oxidation, as supported by SDS-PAGE and DSC results (Figure 3.14 Table 3.10). In addition, this increase in protein solubility might be caused by modifications of chemical groups especially sulfhydryls during the storage period, whereas, cryoprotectants or cryoprotectants and antioxidant treatments maintained a higher high protein

solubility than the MPI without cryoprotectants or cryoprotectants and antioxidant treatments during frozen storage. This supports the ability of sucrose/sorbitol to reduce protein denaturation during frozen storage (Jittinandana and others, 2003).

WHC is known to indicate the ability of a protein gel to retain water against a gravitational force. It reflects the extent of denaturation of the protein. Water content in this study for all treatments did not differ significantly *(P>0.05)* from the beginning to the end of frozen storage.

Table 12- Shows the emulsification capacity and oil holding capacity of myofibrillar proteins stored at -18°C for 90 days. Emulsification is one of the most important functionality in the manufacturing of many formulated foods. Emulsion represents a heterogeneous mixture of fat globulars. Food emulsion can be of the oil in water (O/W) or water in oil (W/O) type. Both EC and OHC of MPI for most of the treatments did not differ significantly (*P>0.05*) in the entire frozen storage period. This observation suggesting storage period did affect protein functionality. Therefore frozen storage (-18°C) itself has a great influence on protein functionality.

3.3 .7 Summary

The present study reveals that, improvement in the functional properties of silver carp MPI were greatly influenced by cryoprotectants and antioxidants, this reflect a great role played by sucrose/ sorbitol and antioxidants in protecting native protein structure to a greater extent owing to impact of frozen storage and oxidation respectively. In addition, cryoprotectants decreased myosin susceptibility to thermal denaturation compared with MPI stored without cryoprotectants. This conclusion is valid based on DSC and SDS-PAGE results. Moreover, protein carbonyl content and dityrosine formation results clearly show that antioxidants (with the exception of ascorbate which caused increase of carbonyl content during storage) protected silver carp MPI against protein oxidation during storage. Therefore, to maximize the functionality of muscle proteins during silver carp storage and processing to enhance its utilization as a low value fish that is susceptible to oxidation, it is necessary to include antioxidants to minimize protein oxidation.

Table 11 – The effect of cryoprotectants and antioxidants on solubility and water holding capacity of silver carp MPI stored at -18°C for 90days [1]

Frozen Storage Time (days)	Solubility (%)					Water holding capacity(mL/g)				
	MPI	MPI+S	MPI +S+A	MPI+S +E	MPI+S+A+E	MPI	MPI+S	MPI +S+A	MPI+S +E	MPI+S+A+E
3	92.2 ± 2.24^{c}	92.8 ± 2.07^{c}	ND*	ND*	ND*	6.0 ± 0.28^{a}	7.4 ± 0.37^{a}	ND*	ND*	ND*
7	88.8±1.19^{c}	93.7±1.17^{c}	ND*	ND*	ND*	5.8 ± 0.63^{a}	7.2± 0.09^{a}	ND*	ND*	ND*
14	82.4 ± 3.69^{b}	83.5 ± 0.80^{b}	ND*	ND*	ND*	5.3 ± 0.64^{a}	6.8 ± 0.09^{a}	ND*	ND*	ND*
30	77.9±0.58^{ab}	83.3±0.7^{b}	87.8 ±1.78^{a}	88.7± 2.52^{b}	84.5 ± 2.69^{a}	5.4 ± 0.62^{a}	6.5 ± 0.43^{a}	6.1 ± 0.44^{a}	7.5 ± 0.43^{a}	7.0 ± 0.77^{a}
60	76.7 ± 0.53^{a}	77.2±0.58^{a}	84.1 ± 1.69^{a}	86.2 ± 1.86a^{b}	82.49 ± 2.25^{a}	6.0 ± 0.17^{a}	6.9 ± 0.63^{a}	6.1 ± 0.48^{a}	7.6 ± 0.52^{a}	7.0 ± 0.50^{a}
90	74.6 ± 0.12^{a}	76.7 ± 0.25^{a}	85.2± 2.33^{a}	85.6± 3.46^{a}	83.71± 2.13^{a}	5.1 ± 0.14^{a}	6.5 ± 0.05^{a}	5.1 ± 0.50^{a}	6.5 ± 0.46^{a}	6.2 ± 0.53^{a}

[1]Means (3 replications, n = 3) in the same column without a common letter differ significantly ($P < 0.05$). ND* not determined

Table 12 - The effect of cryoprotectants and antioxidants on emulsification capacity and oil holding capacity of silver carp MPI stored at -18°C for 90days [1]

Frozen Storage Time (days)	Emulsification capacity (mL/g)					Oil holding capacity (mL/g)				
	MPI	MPI+S	MPI +S+A	MPI+S +E	MPI+S+A+E	MPI	MPI+S	MPI +S+A	MPI+S +E	MPI+S+A+E
3	26.5± 0.99^{a}	27.5± 0.99^{a}	ND*	ND*	ND*	0.8+0.09^{a}	0.8 ± 0.09^{a}	ND*	ND*	ND*
7	26.8 ± 0.19^{a}	27.4 ± 0.90^{a}	ND*	ND*	ND*	1.1±0.07^{b}	1.2±0.08^{b}	ND*	ND*	ND*
14	27.4 ± 0.89^{a}	30.1 ± 2.23^{a}	ND*	ND*	ND*	1.1 ± 0.05^{b}	1.2± 0.02^{b}	ND*	ND*	ND*
30	28.0 ± 0.38^{a}	29.6 ± 1.12^{a}	25.3 ± 1.00^{a}	26.50 ± 1.323^{b}	28.1± 0.70^{a}	1.1± 0.08^{b}	1.1± 0.06^{b}	0.5 ±0.01^{a}	0.5±01^{a}	0.6 ±0.01^{a}
60	28.3 ± 0.65^{a}	30.1± 1.37^{a}	24.3 ± 0.40^{a}	23.7 ± 1.15^{a}	26.4 ± 1.18^{a}	1.2± 0.07^{b}	1.2± 0.02^{b}	0.4± 0.02^{b}	0.52 ±0.13^{a}	0.6 ± 0.13^{a}
90	28.20 ± 0.61^{a}	30.8± 0.90^{a}	23.8± 0.51^{a}	23.7 ± 0.40^{a}	26.2 ± 0.95^{a}	1.1 ±0.02^{b}	1.2 ± 0.06^{b}	0.3± 0.02^{c}	0.4 ± 0.02^{a}	0.5 ± 0.13^{a}

[1]Means (3 replications, n = 3) in the same column without a common letter differ significantly ($P < 0.05$). ND* not determine

4.0 GENERAL CONCLUSION AND RECOMMENDATIONS

4.1 General conclusion

Fish species differ in their susceptibility to protein denaturation and aggregation during frozen storage, in this study silver carp was prepared in three forms (beheaded whole fish, fish mince and myofibrillar protein isolate) for frozen storage. The susceptibility of muscle foods to oxidative processes stems from their relatively high concentrations of unsaturated lipids, heme pigments, metal catalysts, and also processes such as comminuting and grinding, are known to facilitate oxidation which often lead to alterations in muscle protein functionalities, with this regard Fenton oxidation model system was used to study changes of protein functionality, which gave a general view of changes taking place in frozen storage with respect to metal catalyzed protein oxidation. In our present study on frozen storage and oxidation the following can be deduced:

- Changes in the functional properties of silver carp MPI were greatly influenced by loss of MPI solubility, which reflect, the changes in the native protein structure to a greater extent owing to impact of iron-catalyzed oxidation system.
- An iron-catalyzed oxidation system has impact on functional changes of processed white muscle fish.
- The reduced protein functionality can be related to the changes in the chemical and physical properties (structural change) of oxidized and denatured proteins as confirmed by SDS-PAGE, and GPC results. Therefore, to maximize the functionality of muscle proteins during silver carp processing and storage, incorporating antioxidants in samples prepared for frozen storage was the best option to minimize protein oxidation.
- Whole fish frozen storage was more susceptible to oxidation compared to fish mince and myofibrillar protein isolate due to their different lipid content.
- Frozen storage of silver carp fish mince and myofibrillar protein isolate at -18°C caused a small but significant decrease in the functionality.
- Improvement in the functional properties of silver carp MPI were greatly influenced by cryoprotectants and antioxidants, this reflect a great role played by sucrose/ sorbitol and antioxidants in protecting native protein structure to a greater extent owing to impact of frozen storage and oxidation respectively.

- ❖ Cryoprotectants decreased myosin susceptibility to thermal denaturation compared with MPI stored without cryoprotectants.
- ❖ The addition of sodium chloride to 0.6M at pH6.5 improved protein functionality of frozen stored MPI.

4.2 Recommendations

- ❖ The interaction of protein and lipid or lipid oxidation products during frozen storage is worthy studying.
- ❖ Long storage time, the freezing temperature, and the rate of freezing, vacuum packaging or packaging materials, are worthy studying to further explore silver carp storage.
- ❖ Changes in the functional properties during processing and storage need further investigations with respect to quality of protein in the muscle foods.
- ❖ Application of frozen stored MPI on preparation of comminuted meat and fish products for instance sausages, kamaboko or fish ball with intention of evaluating binding properties is also worthy studying.

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APPENDIX

STANDARDS-Molecular weights and their retention time

660 kDa 8.820 min

200 kDa 10.433 min

150 kDa 10.611 min

66 kDa 10.964 min

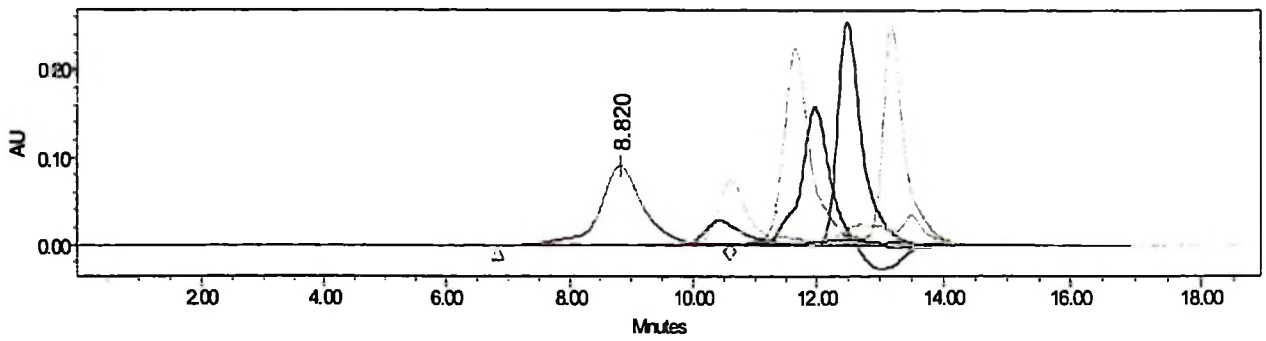
29 kDa 11.654 min

12 kDa 11.968 min

6.5 kDa 12.488 min

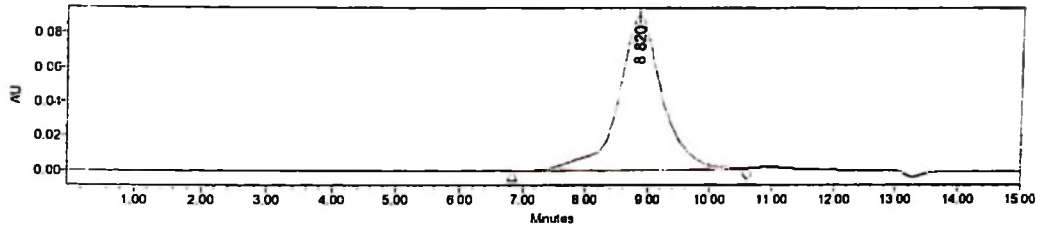
1 kDa 13.193 min

Combined peaks for stds

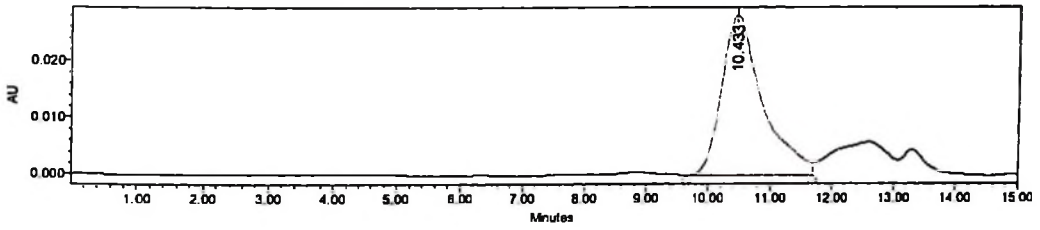


Appendix

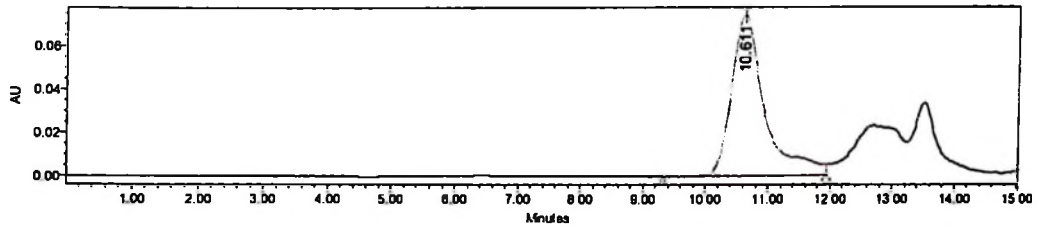
660 kDa 8.820 min



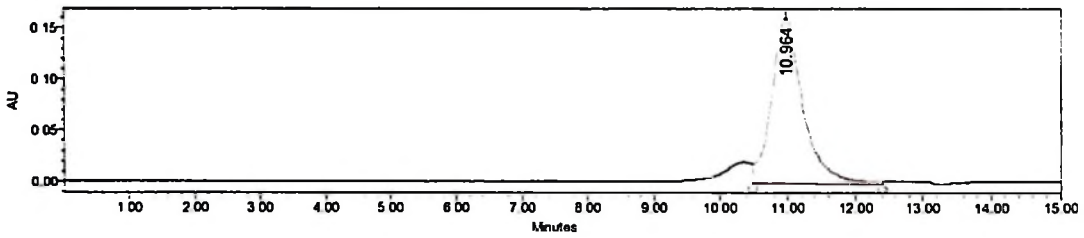
200 kDa 10.433 min



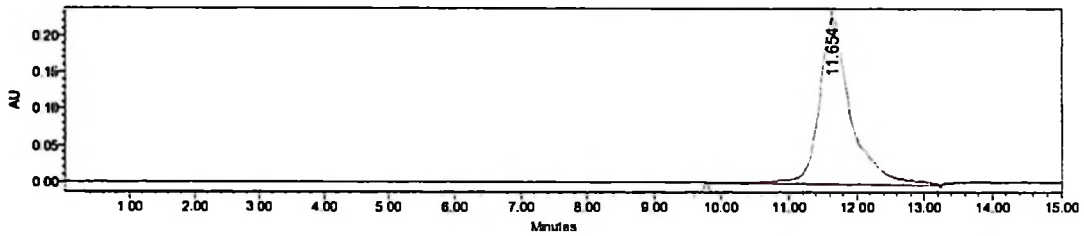
150 kDa 10.611 min



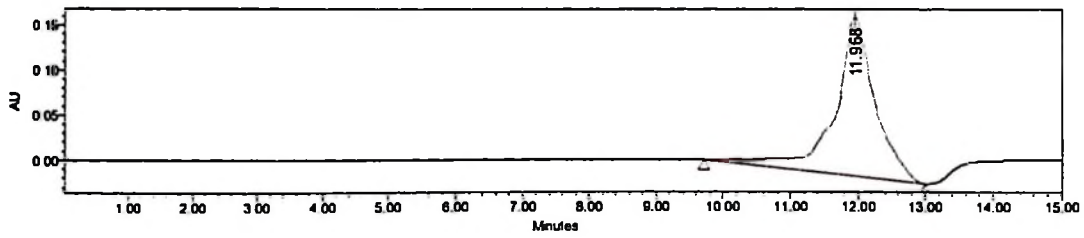
66 kDa 10.964 min



29 kDa 11.654 min

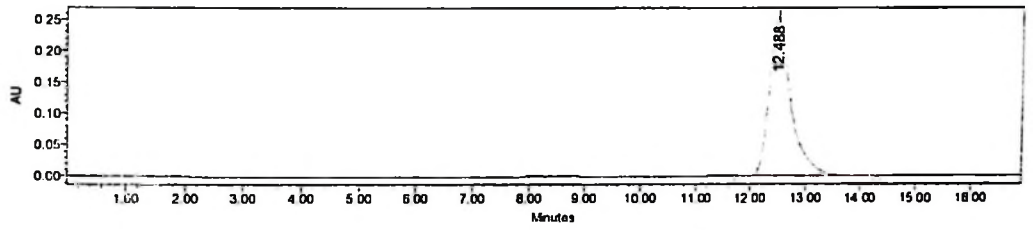


12 kDa 11.968 min

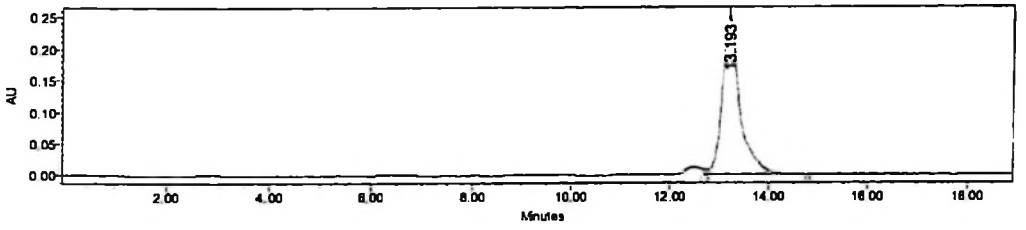


Appendix

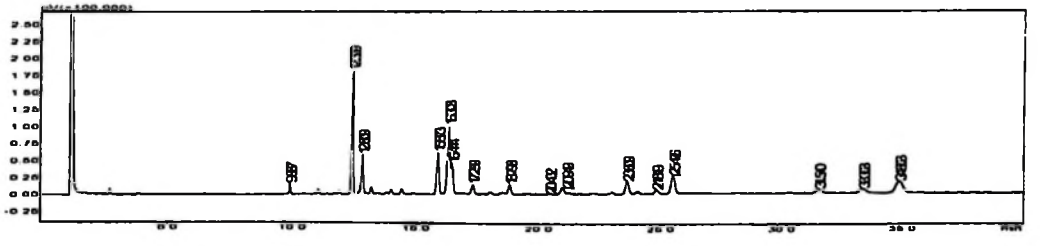
6.5 kDa 12.488 min



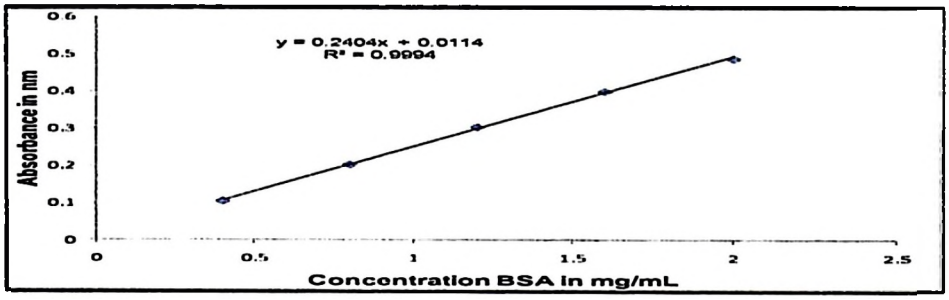
1 kDa 13.193 min



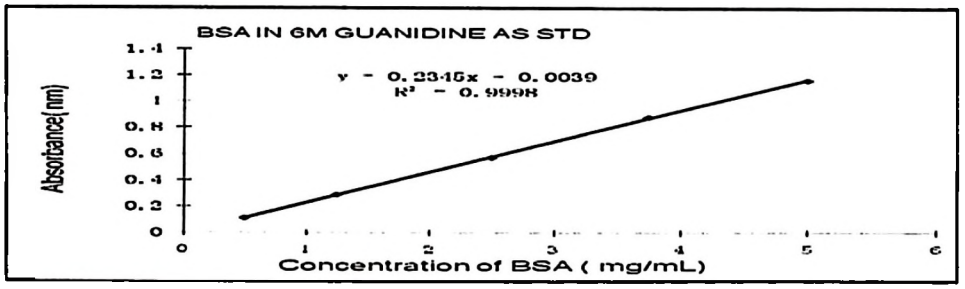
Fatty acid profile of Silver carp MPI



Biuret Standard curve

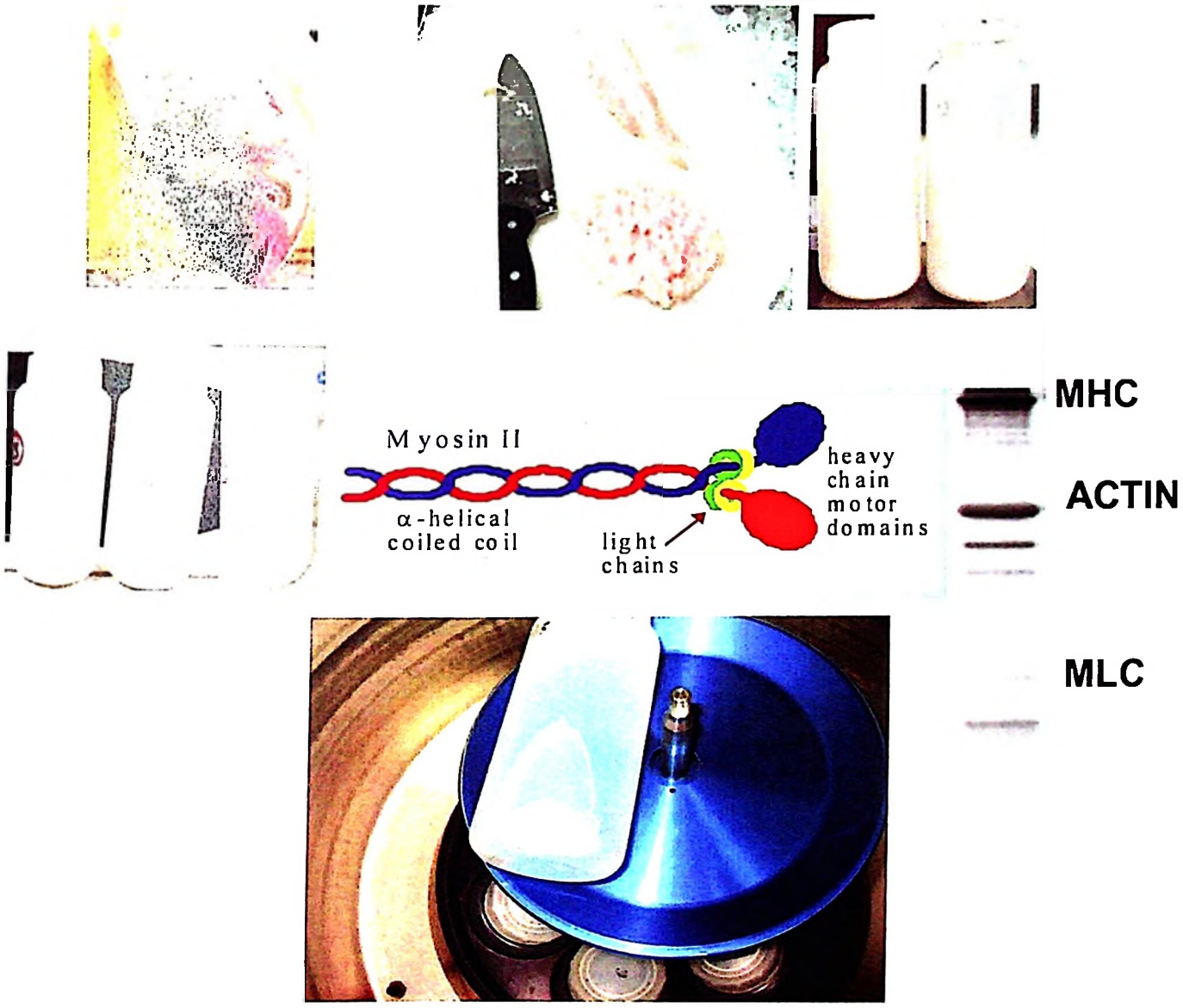


BSA in 6 M guanidine as standard curve



Appendix

Myofibrillar protein isolate



Frozen storage of silver carp beheaded fish, fish mince and silver carp myofibrillar protein isolates

PAPERS PUBLISHED

1. Erasto Mlyuka, Jie Chen. (2010). Protein Oxidation of Silver Carp Muscle During a Freezing Process "***China Journal Net," of data - Digital Periodicals.*** (accepted for publication).

2. Erasto Mlyuka, Youling L. Xiong, Jie Chen (2010). Susceptibility of Silver Carp (*Hypophthalmichthys molitrix*) Myofibrillar Protein to Iron-catalyzed Oxidation and Resulting Functionality Changes. ***Journal of Food Science.*** (under review).