CHAPTER 2

REVIEW OF LITERATURES

2.1 Compositions and characteristics of fatty and dark-flesh fish

Fatty and dark-flesh fish species currently make up 40-50% of the total fish caught in the world and most of them are still considered as underutilized fish (Gunning, 1997; Okada, 1980). Main characteristics of those fish, which limit their use, are high lipid content, less stable proteins, high concentration of heme proteins, low ultimate pH, and high proteolytic activity. These attributes are responsible for inferior quality and poor stability of the fish and their products (Whittle et al., 1990). Consequently, the increasing interest and effort have been paid to improve the quality and to utilize those fish, especially for human foods. Okada (1980) proposed that use of those fish for surimi production would be an appropriate way to achieve that intention since the extensive washing is able to remove water-soluble substances such as sarcoplasmic proteins and other soluble proteins. The components lowering the product quality would therefore be eliminated (Tseo et al., 1983). It is possible to make surimi with good gelling ability from dark-flesh fish if the fish is fresh. However, due to many limiting factors, the surimi production from those species is still far from success based on both economic perspective and consumer viewpoint. The most important problem associated with producing surimi from the dark flesh fish is the high content of red pigments (Kristinsson and Hultin, 2004).

Generally, migratory fish require energy for long-term exercise supplied by the oxidative metabolism within dark muscle. These fish contain the dark muscle about 10%, whereas lean and white-flesh fish have less than 3% (Shimizu et al.,

1992). Diameter of red muscle cell is generally much smaller than that of white muscle cell. To meet the oxygen demand of each single cell within the dark muscle, there are much higher numbers of capillary vessels within the red muscle (Bone, 1978). Oxygen is a very strong oxidizing agent and there are many free radicals produced by biological system with a significant role of oxygen (Shikama, 1998). As a result, dark muscle is prone to oxidation.

The pelagic fish contain high amount of lipid which can vary with seasons (Bandarra et al., 1997; Okada, 1980). This has a potent impact on its stability and processing. In the standard surimi processing, removal of the lipid relies primarily on washing of fish mince. This process could remove 30-80% of initial lipid content (Barrero and Bello, 2000; Roussel and Cheftel, 1988). Polar lipid of their membrane system has been found as a major contribution on oxidation. The lipid not only contains high portion of highly polyunsaturated fatty acids but also has a very large surface area due to physical structure of membrane. These cause the lipid susceptible to oxidation (Hultin, 1995).

The dark muscle of pelagic species contains higher amount of sarcoplasmic proteins than the white muscle. It is generally accepted that these proteins cause the detrimental effect on surimi gelation by binding to functional proteins, myofibrillar protein (Shimizu et al., 1992). One objective of washing in surimi processing is therefore to remove the sarcoplasmic proteins with a consequent concentration of the myofibrillar protein (Hultin and Kelleher, 2000).

2.2 Surimi processing for the fatty and dark-flesh fish

For the standard surimi processing, fish mince is washed three times with water using a fish mince to water ratio of 1:3. Washed mince is partially dewatered

after each exchange. To aid a final dewatering, a 0.2% NaCl solution, instead of water, is recommended for a third washing. Dehydrate-washed mince is then passed through a refiner or a strainer to remove any fish bone, skin, and connective tissue residue. Finally, a screw press is commonly used for a final dewatering. The purified mince is subsequently mixed with cryoprotectants before freezing as the last step (Lee, 1984). The goal of making surimi is to produce industrial-purified fish myofibrillar proteins with high functional properties, especially a gelling property. In addition, surimi is required to have the light or white color, mild fish odor, and frozen stability. All these quality characteristics are hardly met by dark-flesh fish surimi prepared by the standard process. However, high gelling ability surimi could be produced from highly fresh pelagic fish (Leinot et al., 1992). Attempts therefore have been made to modify the procedures of the standard process to effectively produce surimi from the dark-flesh fishes.

Most of undesirable compounds and pigments are water removable. To improve the stability, functionality and whiteness of the dark-flesh fish surimi, modification of the washing procedure has been focused (Tseo et al., 1983; Shimizu et al., 1992; Lee, 1984). A straightforward way to improve color values of surimi is to adjust the washing conditions to enhance pigment extractability such as washing time, washing cycle, and/or water quality (Lin and Park, 1996; Ramirez-Suirez et al., 2000; Gwinn, 1992). However, optimization of these factors was not able to eliminate the pigment to produce surimi as white as the surimi prepared from the lean, white-flesh species (Barrero and Bello, 2000; Roussel and Cheftel, 1988; Hennigar et al., 1988). Furthermore, extended washing period would result in high hydration of mince and degradation of myofibrillar proteins, making the subsequent dehydration process more

difficult and could reduce the gel forming ability of the surimi (Lin and Park, 1996; Sonu, 1986; Okada, 1980; Lee, 1986).

2.2.1 Alkaline washing

Adjusting pH of fish mince to alkaline pH by washing with alkaline solutions was the approach used for obtaining an improved functional property of surimi from the pelagic fish. A low-pH muscle of the post mortem fish may be neutralized by alkalinity of the washing media, and in turn, the denaturation of muscle protein and progressive rate of lipid oxidation are decreased. Furthermore, efficiency of lipid removal by washing at high pH was also higher than that of washing with water (Shimizu et al., 1992). Alkaline phosphate or bicarbonate buffers effectively removed the pigments from red meats (Hernandez et al., 1986; Dawson et al., 1988). These solutions at various concentrations successfully enhanced pigment removal from fish mince (Barrero and Bello, 2000; Chen et al., 1996; Roussel and Cheftel, 1988). Washing sardine mince three times with 0.5% $NaHCO_3$ improved color values of the sardine surimi significantly, compared with those of surimi prepared by washing only with water (Barrero and Bello, 2000). However, it is still difficult to draw a conclusion whether efficiency of the procedure brought about either by changing of pH or by the solvents used or by both parameters. Alkaline treatment alone did not effectively improve the color of surimi of pelagic fish (Jiang et al., 1998). In addition, washing fish mince at higher pH increases solubility of muscle protein and water holding capacity of fish mince with a subsequent low protein recovery and a difficulty on dewatering, respectively (Shimizu et al., 1992).

Loss in soluble muscle protein due to washing at high pH may favor the co-extraction of other proteins such as hemoglobin and myoglobin or may reduce a protein(s) controlling extractability of heme pigment. This latter possible mechanism

was proved in mackerel and chicken breast muscle by Hultin et al., (1995). Certain proteins had to be removed before the remaining myofibrillar protein could be solubilized in water. The another most obviously probable mechanism may be due to a minimization of heme proteins alteration at low pH (Binotti et al., 1971). Both hemoglobin and myoglobin are not stable if pH is below 7. Peroxidation initiated by heme proteins was increased as pH was lower than a physiological value (Richards et al., 2002a,b).

2.2.2 Washing with ozone

Ozone (O_3), a strong oxidation reagent, is capable of decoloration (Bailey, 1982). It has been successfully applied for decoloration of swine hemoglobin powder (Chang et al., 1995) due to the destruction of the porphyrin of heme proteins (Buckley et al., 1975). Jiang et al. (1998) proved that ozonation of mackerel mince for 30 min could improve whiteness of surimi significantly, compared to that of surimi prepared by an alkaline washing. The technique, however, decreased gelling ability of an obtained surimi.

2.2.3 Uses of masking agents

The use of whitening agents such as titanium dioxide, vegetable fat based agents, hydrophilic colloids such as milk, gum hydrocolloids, mixture of sugars, surfactants and fats, to whiten fish mince or surimi have also been investigated (Ravichander and Keay, 1976; Grantham, 1981). Meacock et al. (1997) developed a successful whitening procedure for fish mince using titanium dioxide. An application of 0.1% titanium dioxide in catfish significantly improved whiteness and consumer acceptance of catfish surimi (Prinyawiwatkul et al., 2002).

2.3 The novel surimi processing

Recently, a new approach for obtaining functional protein from dark-flesh fish has been developed at the University of Massachusetts Marine Station. A typical process would be carried out in the following way. Fish muscle is homogenized at 1 part of muscle to 9 parts of water. The pH is adjusted to 11 by using NaOH to solubilize proteins. The mixture is then centrifuged. Thus, lipid fractions, light oils and lipid membrane, are separated from the main protein solution due to density difference. The soluble protein in supernatant is then precipitated by adjusting pH to 5.5 and collected by centrifugation. The sediment or protein isolate can be adjusted to a neutral pH with the addition of bicarbonate and the cryoprotectants are added before freezing (Hultin and Kelleher, 1999, 2001).

The process reduces the limitations of surimi production from the pelagic fish, compared with the standard process. It also provides advantages over the conventional one even though their degree may vary by the species of fish used. Although, the amount of water used in the process is comparable with that generally recommended for the surimi industry used, the novel process offers a better leaching operation (Hultin and Kelleher, 1999; 2001; 2002). Homogenization of fish muscle allows a rapid and high efficient protein extraction. For example, a 20-min extraction of ground mackerel muscle by washing procedure performing in surimi processing yielded less than 80% extractable muscle protein (Shimizu et al., 1992). The color of the protein prepared by the new process was much better than that color obtained by the standard procedure as indicated by the lowering of “b” value (Kelleher et al., 1994). Dilution of muscle proteins with the amount of water decreases viscosity of the protein solution to workable level. This also, in part, allows separation of light lipid and membrane lipid from the protein solution by the first centrifugation (Hultin and

Kelleher, 2000). Lipid reduction of the mackerel and catfish mince increased from 72.1 and 58.3 % to 79.1 and 88.6%, respectively, if the standard surimi processing was substituted with the alkaline solubilization process. The alkaline process also improved protein recovery. Protein recovery of mackerel and catfish surimi produced by the standard process was 54.1 and 62.3 %, respectively, and increased to 69.3 and 82.1% when the alkaline solubilization process was used (Kristinsson, 2003).

2.4 Hemoglobin

2.4.1 Structure function and properties

Hemoglobin and myoglobin are two of the most important proteins of vertebrates. Hemoglobin is the major heme protein of red blood cells and is responsible for the transportation of oxygen to the tissues while myoglobin is the oxygen container of cell (Perutz, 1990). Hemoglobin consists of four polypeptide chains, two of α chains and two of β chains. The four chains are held together by noncovalent attractions. Each α chain is in contact with β chains ($\alpha_1\beta_1$ and $\alpha_2\beta_2$). Conversely, there are few interactions between the two α chains or between the two β chains (Baldwin and Chothia, 1979). The conformation of the α and β subunits differs only by addition of one helix (D) in the β subunit. The three-dimentional structures of the α and β chains of hemoglobin and myoglobin are similar even though they are quite different in amino acid sequence. Differences in number and identity of amino acids have been reported in hemoglobin from different sources (Perutz, 1990; Jensen et al., 1998).

Capacity of hemoglobin or myoglobin to bind oxygen depends on the presence of the nonpolypeptide unit namely a heme group. The heme buried in hydrophobic pocket of hemoglobin consists of an organic part and an iron atom (Figure 2-1)

(Dickerson and Geis, 1986). The organic part, protoporphyrin, is made up of four pyrrole rings. The four pyrroles are linked by methene bridges to form a tetrapyrrole ring. Four methyl, two vinyl, and two propionate side chains are attached to tetrapyrole ring. These substituents can be arranged in fifteen different ways. Only one of these isomers, called protoporphyrin IX, is present in biological systems (Perutz, 1970). The iron atom in heme binds to the four nitrogens in the center of the protoporphyrin ring. The iron can form two additional bonds, one on either side of the heme plane. These binding sites are termed the fifth and sixth coordination positions. At the fifth coordination position, the iron is directly bonded to histidine of globin called the proximal histidine while the sixth coordination position is the oxygen-binding site. The iron atom can be in the ferrous (+2) or the ferric (+3) oxidation state, and the corresponding forms of hemoglobin are called ferrohemoglobin and ferrihemoglobin, respectively. Ferrihemoglobin is also called methemoglobin where water molecule occupies the sixth coordination. Only ferrohemoglobin, the +2 oxidation state, can bind oxygen. Therefore, most hemoglobin molecules are found either with no oxygen (deoxyhemoglobin, unliganded Hb) or with four oxygens (oxyhemoglobin, liganded Hb) (Fermi et al., 1984).

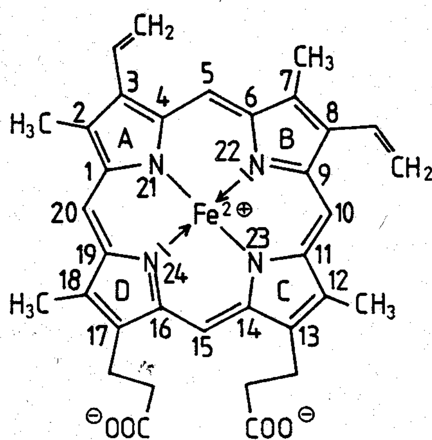


Figure 2-1 Protoporphyrin IX

Source: Stryer (1988)

Binding of oxygen in hemoglobin is cooperative. This means that binding at one heme facilitates binding of oxygen at other hemes on the same molecule (Jensen et al., 1998). Oxygen binding property of hemoglobin is also affected by pH. In the physiological range, a lowering of pH decreases the oxygen affinity of hemoglobin. Increasing the concentration of CO_2 also lowers the oxygen affinity. These linkages between the binding of O_2 and concentration of H^+ and CO_2 are known as the Bohr effect (Riggs, 1988). Hemoglobin of some fish expressed a large decrease in both oxygen affinity and cooperative at low pH. This characteristic of hemoglobin is known as the Root effect (Brittain, 1987). Typically, the Bohr effect expresses its role when blood pH drops from about 7.4 to 6.5 (Stryer, 1988). A further decrease in blood pH is considered the Root effect (Manning et al., 1998).

Many fish species have multiple hemoglobin components, which are characterized by significant differences in amino acid sequence and in functional properties (Brunori et al., 1975; Brunori, 1975). This multiplicity is in contrast to human hemoglobin which has a single main hemoglobin. Based on the multiplicity, teleost fish fall into three major classes: class I, II and III (Weber et al., 1976). The class I hemoglobin as in carp is an electrophoretically anodal hemoglobins that have Bohr, Root, phosphate, and temperature effects (Jensen et al., 1998). The class II hemoglobin as in trout and eel consists of both anodal components and cathodal hemoglobins that exhibit high oxygen affinity and small Bohr effect and low temperature sensitivities (Zolese et al., 1999). The class III hemoglobin as in tuna is hemoglobins that is sensitive to pH but insensitive to temperature (Rossi-Fanelli, 1960).

Light absorption is one of basic properties of heme proteins. Consequently, changes in their spectral absorption characteristics are regularly assessed to monitor stability/change of the proteins. The conjugated double bond system of the porphyrin ring causes the strong absorptions of the heme proteins, terms the α, β, and γ bands. The typical absorbance spectra of hemoglobin and its derivatives are shown in Figure 2-2. Typically, α bands occur at the longest wavelengths (550 to 650 nm), γ bands at the shortest wavelengths (also called Soret bands), and β bands lie between (Poole and Kalnenieks, 2000).

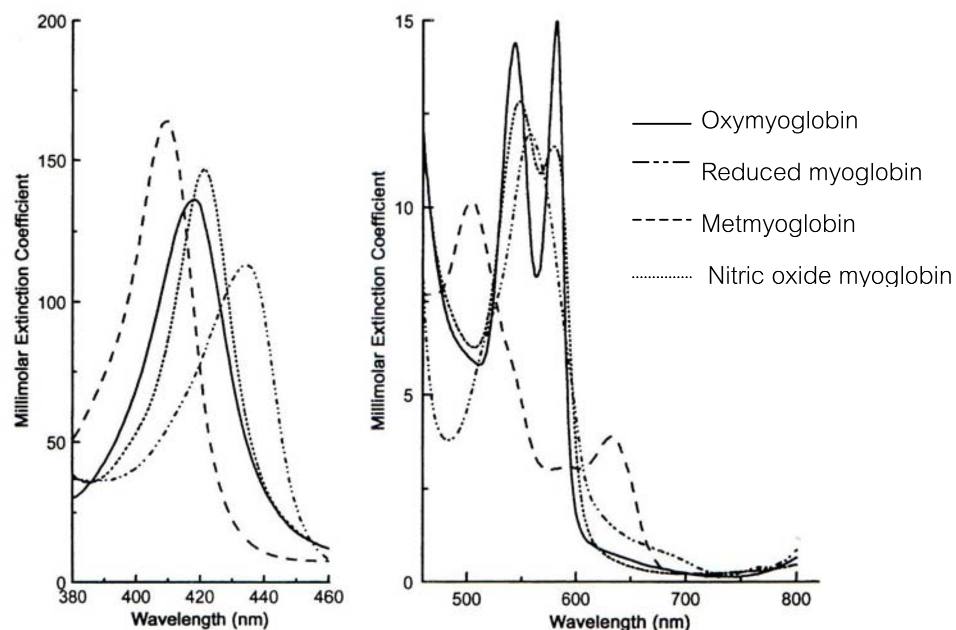


Figure 2-2 The typical absorbance spectra of heme protein and its derivatives
Sourec: Millar et al. (1996)

Several changes of hemoglobin are associated with its absorbance spectrum. For instance, the Soret absorbance of human hemoglobin shifted from its starting value of 414 nm to 410 nm was characteristic of hemichrome (Akhrem et al., 1989;

Marva and Hebbel, 1994). The difference in absorbance at peaks of α and β bands, reflected degree in oxygenation of hemoglobin (Pelster and Weber, 1991), was used for monitoring oxygenation of hemoglobin upon changing of pH. It was found that relative oxygenation of trout hemoglobin decreased abruptly when pH was changed from about 7.5 to 6.5 as shown in Figure 2-3 (Richards et al., 2002a,b; Richards and Hultin, 2000;).

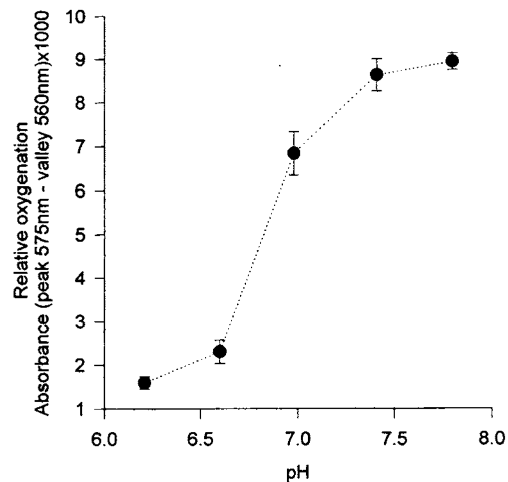
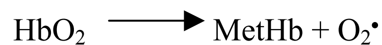


Figure 2-3 Relative oxygenation of trout hemoglobin
Source: Richards and Hultin (2000)

2.4.2 Reaction of hemoglobin

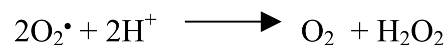
Autoxidation

Autoxidation is the spontaneous oxidation of hemoglobin to methemoglobin by molecular oxygen. Oxyhemoglobin is known to undergo a slow, but spontaneous intra-molecular oxidation-reduction reaction, in which the heme iron is oxidized to the ferric form and the oxygen is reduced to superoxide ($O_2^{\bullet}$) (Everse and Hsia, 1997):



$$HbO_2 \longrightarrow MetHb + O_2^{\bullet}$$

Within the erythrocyte, this reaction has no biological consequences, because the superoxide is rapidly dismutated to oxygen and hydrogen peroxide by superoxide dismutase (Everse and Hsia, 1997):



$$2O_2^{\bullet} + 2H^{+} \longrightarrow O_2 + H_2O_2$$

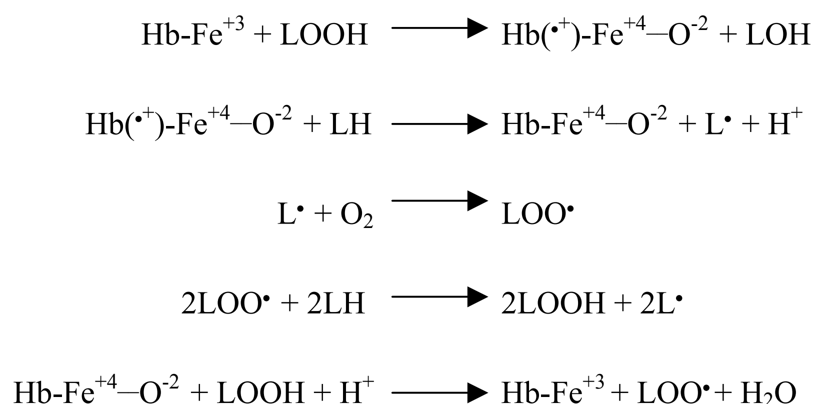
Furthermore, within the erythrocyte, any methemoglobin is reduced back to the ferrous form by methemoglobin reductase (Renerre and Labas, 1987). Any hydrogen peroxide is converted to oxygen and water by catalase, or utilized for oxidation of glutathione by glutathione peroxidase (Baron and Andersen, 2002; Shikama, 1998; Jensen et al., 1998). It is, however, common to find that hemoglobin obtained from freshly drawn blood contains significant amounts of the ferric derivative, i.e., 5% or more (DeYoung et al., 1994).

Among the factors influencing the rate of autoxidation of myoglobin and hemoglobin, the effect of pH was widely investigated (Wallace et al., 1982). The results clearly showed that the rate of the autoxidation increased with increasing hydrogen ion concentration (Tsuruga and Shikama, 1997; Mansouri and Winterhalter, 1973). Therefore the post mortem processes continuously depress muscle pH, progressively inactivate the reductive enzyme systems and stimulate acid-catalyzed

autoxidation of heme proteins. Oxygenation state of heme proteins affected the reaction and hemoglobin with low oxygen affinity was susceptible to a rapid autoxidation rate (Astatke et al., 1992). The kinetic study on the oxidation of sperm whale oxymyoglobin with H_2O_2 as the oxidant revealed that H_2O_2 can oxidize the deoxy species more than 100 times greater than oxidized oxymyoglobin (Yusa and Shikama, 1987).

Lipid peroxidation

Intact hemoglobin, not free heme, was responsible for increasing rate of the lipid peroxidation (Gutteridge, 1987). Several reaction schemes were proposed by Kanner and Harel (1985). These involve the withdrawal of an electron from an unsaturated fatty acid or a fatty acid hydroperoxide by the ferry-hemoglobin. The reactions result in the formation of fatty acid radicals. In the presence of oxygen, a chain reaction is catalyzed, leading to extensive reactions as follows (Everse and Hsia, 1997; Kanner and Harel, 1985):



(LOOH: fatty acid, LOH:fatty acid hydroperoxide, $L^{\bullet}$ and $LOO^{\bullet}$: radical species)

Other pathways of lipid oxidation mediated by hemoglobin include the action of hemin or iron released from degradation of heme proteins (Richards and Hultin, 2000; Avissar and Shaklai, 1984).

Hemoglobin stimulated the reaction slightly at the physiological pH of 7.2, but the rate increased considerably at pH 6.5 (Gutteridge, 1987). Richards and Hultin (2000) also reported an increasing lipid oxidation rate with concomitant decrease in oxygenation of the hemoglobin when pH was reduced from 7.5 to 6.5. Richards et al. (2002) further reported that deoxyhemoglobin was more pro-oxidative than its oxygenated form at physiological pH of fish muscle. Kanner and Harel (1985) stated that effective lipid peroxidation induced by hemoglobin requires the presence of hydrogen peroxide as little as 30 μM.

Aqueous phase of fish muscle consists of many pro-oxidants and antioxidants (Decker and Hultin, 1990). Several endogenous components are known to interact to heme proteins and their derivatives to serve several biological functions. Haptoglobin, for instance, is a blood protein that binds free hemoglobin involved in the removal of extracellular hemoglobin from circulation (Gutteridge, 1987). Desferroxamine is an iron chelator that has been shown to bind to hemin via the iron moiety (Sullivan et al., 1992). Carnosine can inhibit lipid oxidation promoted by heme proteins (Decker et al., 1995). Divalent ions may also involve indirectly on the binding of heme proteins to membrane. For instance, Ca^{2+} forms a tight ion pair with phosphate group of acidic phospholipids, thus shielding its negative charge (McLaughlin et al., 1971).

Binding of hemoglobin and its derivatives to muscle components

In mammalian erythrocytes, hemoglobin binds to membrane components. The most abundant membrane protein is band 3, which presents about 1 million copies per red blood cell. Band 3 consists of a membrane domain, which mediates the

physiological important anion exchange across the membrane, and a cytoplasmic domain, which is anchored to the cytoskelaton to which hemoglobin and glycolytic enzymes also bind (Salhany et al., 1990). Binding of hemoglobin occurs at the N-terminal fragment of the cytoplasmic domain, and deoxyhemoglobin binds more tightly than oxyhemoglobin (Tsunesshige et al., 1987; Walder et al., 1984). Additionally, Marva and Hebbel (1994) proposed that an interaction between hemoglobin and phosphatidylserine liposome, as a membrane model system, involves an initial and reversible electrostatic interaction. This is analogous to the observations of Shaklai et al. (1985) regarding hemoglobin interaction with red blood cell ghost membrane and of Andreyuk and Kisel (1999) involving the protein/lysophospholipid complex. It was proposed that significant adsorption of the proteins to negatively-charged liposomes only occurred at pH values where the number of positive charge moieties exceeds the number of negative charge moieties on the proteins by at least three charge units (Bergers et al., 1993). As well as, it was proposed that electrostatic interaction is likely to be one of the most important forces in determining the way macromolecules interact with membranes. Interaction of hemoglobin with lipid results in a subsequent formation of methemoglobin followed by a rapid transformation of hemoglobin into hemichrome. Thus lipid contact exaggerates the inevitable instability of hemoglobin (Andreyuk and Kisel, 1999).

By using the model system comprising myoglobin and liposome, Bergers et al. (1993) proposed that after initial electrostatic interaction, myoglobin may partially unfold to semirandom coils at the membrane/water interface, which would permit the nonpolar residues to contact the apolar region of the bilayer. Gotz et al. (1994) reported that myoglobin from freshly prepared chicken gizzard, chicken or bovine heart had high fatty acid binding capacity. A 60-70% reduction on the binding

capacity was due to conversion of oxymyoglobin to metmyoglobin. It was therefore proposed that the conformation changes of myoglobin, which is induced by binding of oxygen, favored the interaction of myoglobin with fatty acids.

Hemin, an essential cell constituent as well as a product of hemoglobin denaturation, has been shown to be deleterious to red cell membrane. Chiu et al. (1997) found that hemin has strong binding effect on cell membrane. Its effect is not mediated via lipid peroxidation, but by the interaction of hemin with membrane protein sulhydryl groups.

2.4.3 Changes in fish hemoglobin during storage and processing.

Blood is known to cause deterioration in muscle foods. For this reason, farm animals are generally bled as much as possible when they are slaughtered. Nevertheless, it is never possible to remove all of the blood. Fish are often not bled when they die. In fact, they may have been dead for some time before they are processed. It has been reported that fish blood remains fluid for only about 30 min at temperature just above freezing, after which its clots rapidly (Connell, 1975). Botta et al. (1986) evaluated the effect of two-step bleeding on cod as a function of time on board on grading characteristics that included color and odor of the fillets. Sensory quality was more dependent on time on board prior to bleeding than the actual process used. Bleeding typically removed between 20 and 50% of the blood in whole trout muscle and mackerel light muscle and dark muscle (Richards et al., 2002). Tretsven and Patten (1981) reported that cutting the arteries of rainbow trout reduced the amount of rancidity development after frozen storage for 8 months. Richard and Hultin (2002) found that bleeding of trout and mackerel resulted in increased number of the fishes that had extended shelf-lives. On the other hand, Porter et al. (1992)

found that bleeding did not decrease the rate of lipid oxidation between groups of bled and unbled sockeye salmon as measured by TBARS or loss of specific fatty acids.

Hemorrhages of muscles as induced by different killing techniques and handling procedures may lead to hemoglobin and red blood cells leaving the vascular system. Therefore, it could lead to decrease oxidative stability of muscle (Kranen et al., 2000). Unwashed fillet from fresh mackerel underwent rapid oxidative deterioration compared to unwashed fillet from aged fish. This observation was found having close relation with amount of blood or hemoglobin contaminating the fillet surface (Richards et al., 1998). It was likely caused by increased amount of deoxyhemoglobin, a proposed proxidative heme protein, on the fillet surface upon exposure to ATP/ADP of fresh fish (Richards and Hultin, 2000; Val, 2000).

Pigments concentration and chemical state of the pigments are two of main factors responsible for color of muscle foods (Ledward, 1992). Many ligands can bind to iron atom of heme protein of both hemoglobin and myoglobin and the resultant bonds are responsible for the various colors observed in meat and its products. Relative amount of red muscle in fish not only contributes to fish storage stability but also determines consumer acceptability. Generally, fish red muscle containing high amount of heme proteins appears grayish after heating which is actually a serious deterrent for consumption (Chen et al., 1996). Therefore, there is interest in development of an appropriate means to minimize heme pigments residue in fish muscle. It was, however, founded that all fish hemoglobin are less stable such as more susceptible to autoxidation, denaturation, and precipitation, when compare to other animals (DeYoung et al., 1994). For instance, extractability/solubility of these heme pigments of fishes such as tuna, round herring, and milk fish was steadily decreased during storage either by icing or freezing (Chen et al., 1996; Chow C.J. et al., 1987).

The change was found associated with reduction of physiological pH of the fishes muscle and autoxidation of the heme proteins (Chow C.J. et al., 1987).

At low concentration, tetrameric hemoglobin dissociates reversibly into $\alpha\beta$ dimer. The dissociation constants for the tetramer-dimer dissociation of liganded carp, menhaden, and blue shark hemoglobin are approximately 10^{-8} M (Kwiatkowski et al., 1994). Dissociation of hemoglobin into dimers and monomers increases its susceptibility to and for oxidation (Griffon et al., 1998). It has been suggested that dissociation of hemoglobin tetramers into dimers may enhance the dissociation of heme from the globin protein (Benesch and Kwong, 1995). Consequently, dilution during grinding and washing of muscle can potentially lead to such a dissociation and thus more oxidative problem.

Objectives

1. To study the effect of pH and cod muscle soluble fraction on extractability of cod hemoglobin.
2. To investigate the effect of washing parameters on extractability of cod hemoglobin.
3. To examine the binding of cod and herring hemoglobin to cod and herring sarcoplasmic reticulum.
4. To study the effect of hemoglobin deoxygenation caused by existence of ADP on hemoglobin extractability.
5. To examine the effect of pH values of the alkaline solubilization process and the presence of fish muscle components on extractability of cod hemoglobin.
6. To study the effect of storage and the alkaline solubilization process on extractability of herring heme proteins.

Expected outcomes

The principal assumption of these studies is based on the fact that pH 6.0 is able to induce changes in hemoglobin properties and stability. Exposure of cod hemoglobin to this pH is therefore expected to lower extractability of the hemoglobin. Role of cod muscle soluble fraction on the extractability of hemoglobin under this condition was verified. The treatment enhancing the extractability of cod hemoglobin and herring heme protein was expected to be an outcome from manipulation of the results. Possible relationship between changes of hemoglobin due to the exposure to various pH values and corresponded extractability of the hemoglobin was verified. Results from investigations on binding of the hemoglobin to sarcoplasmic recticulum (SR) and on a relationship between deoxygenation and extractability of hemoglobin caused by existence of ADP can be used to provide supportive explanation on a change of extractable hemoglobin. Improved efficiency on removal of heme protein by the alkaline solubilization process was expected in comparison with that of washing in the standard surimi process. Finally, modification of the alkaline solubilization process, which results in an increase in amount of extractable heme protein and in whiteness of protein isolate as well as the maximized protein recovery, was also expected.

Scope of the research

Washed cod mince was used as a model system to study effect of pH of the mince on extractability of hemoglobin. The effect of cod muscle soluble fraction on the extractability was studied. Salt content and pH of homogenate of washed and unwashed mince was adjusted at extraction in order to investigate their effect on hemoglobin extractability. Effect of pH, cod muscle soluble fractions, and sources of

hemoglobin and SR on binding of hemogloin to SR was tested. Effect of ADP on deoxygenation of cod hemoglobin solution at pH 7.0 and a consequent effect on hemoglobin extractability was examined.

Effect of pH values and a pH treatment on solubility and extractability of cod hemoglobin was examined. Binding between hemoglobin and SR of two species (herring and cod) subjected to the pH treatment was examined. Effect of presence of cod myosin or cod muscle soluble fraction on soluble hemoglobin was studied. Effect of iced storage on extractability of heme protein from herring light and whole muscle was examined. Effect of the alkaline solubilization process with and without pre-washing step on extractable heme protein of the herring whole muscle, color values of protein isolate, and the loss of soluble protein was examined.