CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Cephalopods constitute an important part of the marine resource for human consumption. They are commonly consumed in Mediterranean and Far East, particularly in Japan. Due to their abundance and rapid stock renewal, they are recognized as the most promising resource. Their biological cycle lasts between eight months and two years, depending on the species (Guerra, 1992). In 2001, 34% of total cephalopod catch was taken in Northwest Pacific, 24% in Southwest Atlantic and about 12% in the Western Central. World's cephalopod production in year 2001 consisted of 67% squid (2.2 million tons), 16% cuttlefish and 9% octopus (FAO, 2003). Thailand is an important cephalopod producing country with the fifth production of cephalopod in the world. Japan is the largest producer followed by China, Korea, Argentina and Thailand. Thailand exported frozen cuttlefish and squid for 96490 tons with a value of 15167.7 million baht in year 2004. Japan is the biggest market, whereas Italy and USA are the second and the third biggest market for Thai frozen cuttlefish and squid (Ministry of Commerce Thailand, 2006).

Even though frozen storage is one of preservation methods which prevent microbial spoilage effectively, the chemical deterioration still occurs, depending on conditions of storage. During frozen storage, cephalopod proteins, mainly myofibrillar protein undergo denaturation and aggregation, leading to decreased protein functionality such as water-binding ability, solubility and viscosity (Mutsumoto, 1980; Ueng and Chow, 1998; Moral *et al*., 2000; Ruiz-Capillas *et al*., 2002). So far, frozen cephalopod industries have been facing the decreased

quality of products during frozen storage, especially for frozen cuttlefish. Discoloration sometimes occurs during frozen storage of cuttlefish accompanied by rancid odor. Texture also turns to be toughen and rubbery. This occurrence causes the rejection of products, resulting in the economical loss. It is hypothesized that the contamination of metal ion in cuttlefish and squid during living, catching, post harvest or processing, may contribute to the acceleration of lipid oxidation of cuttlefish and squid. As a consequence, lipid oxidized products such as peroxide, aldehyde, ketone, etc. are formed. Interaction of peroxides or carbonylic peroxide decomposition product with active groups of protein can lead to protein polymerization or aggregation as well as discoloration in cuttlefish and squid.

However, information regarding lipid oxidation, discoloration and physicochemical changes in muscle proteins of cuttlefish and squid especially those are caught in Thailand during extended frozen storage is scarce. The knowledge gained can be supportive for a development on the appropriate method to maintain the prime quality of frozen products. As a consequence, frozen cephalopods take a less risk in a rejection from the consumer or importer. Also, Thai products can be competitive in the world market.

1.2 Literature Review

Cephalopod

Cuttlefish belong to the family Sepiodia, class Cephalopoda, which forms part of phylum Mollusca. Sepioidea is characterized by a thick internal calcium-containing shell called the cuttlebone. Cuttlefish has the length ranging from 2.5 to 90 cm and have a somewhat flattened body bordered by a pair of narrow fins (Figure 1). They feed mainly on crustaceans as well as small fishes. Cuttlefish has been used as human food, as a source of ink, and the cuttlebone is used as a dietary supplement for cage birds. They grow quickly and live in the deeper water. The new adult cuttlefish are ready to spawn at 18-24 months of age. After spawning, the females soon become lethargic and their body quickly deteriorates (Voss *et al*., 1998). The world cuttlefish production increased slightly (+7%) to 530000 tons in 2001. Over the last ten years, the production of cuttlefish has grown by a striking 80%. China, the leading producer with a 60% share of the total cuttlefish production has experienced a further 22% increase in 2001. Chinese cuttlefish has grown strongly since 1990 when it was only 68700 tons. Thailand is an important cuttlefish producer with 64600 tons in 2001, more or less the same as in 1999 but 24% more compared to ten years ago. In year 2001, 325000 tons of cuttlefish was taken in the North West Pacific, representing a 61% of the total and showing a 20% increase compared to that of year 2000 (FAO, 2003).

The long-finned squid *(Loligo peali)* live for less than one year, grow rapidly, and spawn year-round (Figure 2). Most grow to about 12 inches. They swim in large schools during the day but may dig itself a depression in the sand at night to rest. Individuals migrate seasonally, moving offshore during late autumn to over winter in warmer waters along the

edge of the continental shelf and inshore during the spring and early summer (Herkeand and Foltz, 2002). In many countries which are not traditionally cephalopod consumers, the demand for squid is increasing. The best example is the USA, where squid product is very well accepted and well established in fast food chains. Also in other countries with low seafood consumption, such as Argentina, squid has found a market niche in the (fried) fast food sector (FAO, 2003).

Figure1. Cuttlefish (*Sepia pharaonis*)
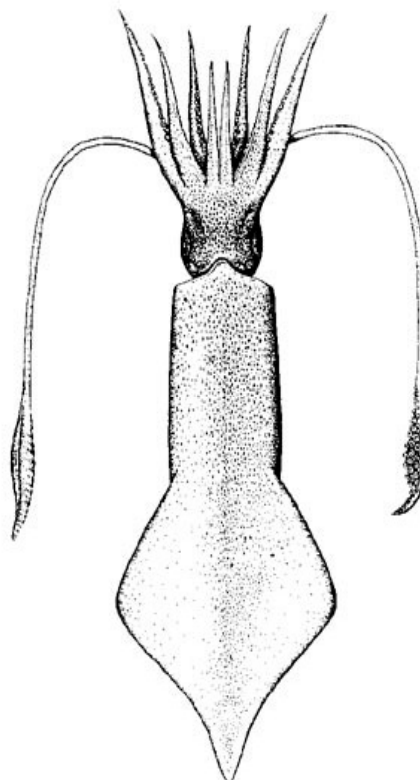Source: Department

Figure 2. Squid (Long finned squid: *Loligo peali*)
Source: FAO (2006)
Tissue structure of cephalopod

The meat of squid is mainly from its mantle. The mantle of squid (*Loligo pealei*) is composed of five layers of tissue (Figure 3) (Otwell and Hamann, 1979). The middle layer contains the muscle tissue with the muscle fibers grouped in bands. The characteristic feature of the squid muscles is their very small size. The elongated cells of myofibril are surrounding a central core which contains sarcoplasmic proteins. The muscle fibers are obliquely striated and covered with a thin sarcolemma. The muscle layer comprises about 98% of the total thickness of the mantle. The muscle is covered on each side by a layer of connective tissue called the outer and the inner tunic. The outer side of most of each tunic is covered by a thin layer tissue, the outer lining, which connects the outer tunic with the skin. It consists of randomly oriented fibers. The inner lining is the inner surface of the mantle (Otwell and Hamann, 1979). The different layers possess different chemical and physical properties, and each layer responds in a specific way to heat treatment. Thus, in addition to the natural structure, technical factors such as cooking time, temperature and cooking method also influence the texture of cooked squid meat (Okuzumi and Fujii, 2000).

In five species of squid, varying in life-style from fast-swimming pelagic predators to sluggish benthic forms, the circular muscle of the mantle was found to be metabolically and structurally differentiated into inner, middle and outer zones. In the middle zone, mitochondrial abundance and the ratio of oxidative to glycolytic enzyme activities were low. This zone

was sandwiched between thinner bands of muscle lining both the inner and outer edges of the mantle. In these bands, mitochondrial abundance and the ratio of oxidative to glycolytic enzyme activity were high. It is proposed that this metabolic differentiation is analogous to the development of red and white muscles in vertebrates and that it serves a similar function, white muscle mainly supporting burst-type swimming and red muscle sustaining steady state oxidation work (Mommsen *et al*., 1981).

Chemical compositions of cephalopod

The chemical composition of squid is dependent on species, growth stage and season. Mantle is the main edible portion of squid constituting about 35-44.7 % of total body (Kreuzer, 1984). Proximate composition of mantle muscle of squid and cuttlefish is shown in Table 1. According to the 4th Amended Japanese Standard Food Content Tables, the edible parts of squid contain 81.8% water, 15.6% crude protein, 1.0% crude fat and 1.5%. The species known as hotaru-ika (firefly or sparkling enope squid) has 78.9% water, 14.2% crude protein, 5.2% crude fat and 0.5% ash (whole body including internal organs) (Okuzumi and Fujii, 2000).
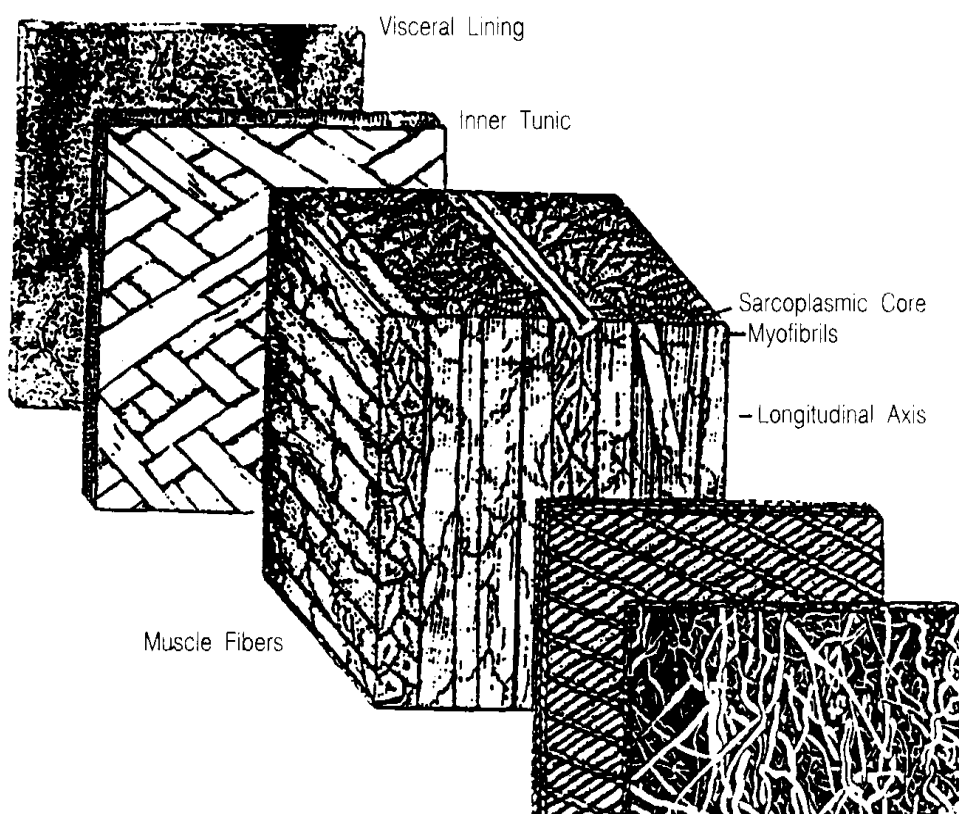
Figure 3. The structure of cephalopod
Source: Otwell and Hamann (1979)

Table 1. Proximate composition of mantle muscle of squid and cuttlefish (%)

Species Ash	Moisture	Protein	Lipid
Todarodes pacificus 1.59	76.6	20.6	1.88
Ommastrephes bartrami 1.71	77.1	20.9	1.33
Nototodarus sloni gouldi 1.78	78.4	18.7	1.66
Nototodarus sloni sloani 1.74	77.1	20.2	1.67
Illex argentinus 1.71	78.6	18.2	2.03
Loligo opalescens 1.52	77.0	18.2	2.74
Sepia esculenta 1.56	81.5	15.6	1.28
Sepia pharaonis 1.86	76.4	20.2	1.36

Source: Kreuzer (1984)

Protein

The type of protein and their functional status are the two factors influencing the texture of cephalopod muscle (Moral *et al.*, 2002). Squid mantle texture is dependent on two structural features (i.e. muscle and connective tissues). Collagen fibers are key factors in longitudinal mantle toughness, whereas muscle fibers are more important transversely (Ottwell and Hamaan, 1979). The main proteins of the muscle are myofibrillar (77-85%), sarcoplasmic (2.0%) and stroma proteins (12-13%) (Okuzumi and Fujii, 2000).

Myofibrillar proteins are the main constituents in squid mantle. Squid protein consists of a thick filament called myosin and a fine filament called actin. The thick filament of squid is formed from a composite core of protein called paramyosin, unique to invertebrates, around which the myosin is coiled in a structure that is unique to squid. As a result, squid has a longer and thicker filament than vertebrate (Okuzumi and Fujii, 2000). Paramyosin was found in the myofibril fraction of the striated muscles with the amounts of 14 % in squid (Sano *et al.*, 1986). Sarcoplasmic proteins are soluble at low ionic strength even water. Although they are fairly stable when frozen, the aggregation can occur in some cases (LeBlance and Gill, 1982). Collagen content of fish and seafood depends on the species, feeding regime, and state of maturity. The structure of collagen fibrils evidently influences the physical properties of the tissue, mainly solubility and texture. The stickiness of the connective tissue depends on species as well as on age, sexual maturity and muscle depletion (Morales *et al.*, 2000). Squid mantle contains about 15% collagen, which is three-fold higher than beef muscle. Fish connective tissue contributes little, if any, to the eating texture of cooked fish. However, some species of squid may develop a tough and rubbery texture upon heat processing (Okuzumi and Fujii, 2000). Mizuta *et al.* (1994) proposed the following three characteristics as collagen-related factors determining texture; (1) total collagen content, (2) distribution or morphology of collagen fibers in muscle and (3)

the content of a specific collagenous component. Morales *et al.* (2000) characterized collagen from some cephalopods (volador: *Illex coindetii*; pota: *Toradopsis eblanae* and octopus: *Eledone* cirrhosa). Collagen content was higher in arm than in mantle for all species. Collagens type I and V were the principal constituents in both anatomical location (arm and mantle) for all species. In general, a higher proportion of type I was isolated than type V, approximately in a ratio 8:1 (type I/type V). Similarly, Mizuta *et al.* (1994) found a type I/type V ratio of 8:1 in mantle of *T. pacificus*.

Cephalopods are fast-growing, reaching sexual maturity in one or two years (Guerra, 1992). Such a short life cycle implies a high rate of protein replacement. Thus, squids (volador and pota) are very active throughout their lifetimes, whereas octopus are highly sedentary, rousing themselves only to pursue their prey. All of this may considerably influence their composition and suitability for processing (Okuzumi and Fujii, 2000). The function of each of the anatomical regions of the live organisms can have a considerable effect on the amount of collagen present and its degree of aggregation. The arms perform a grasping function and therefore have to withstand more strain than the mantle. The squid mantle needs to be more elastic to perform its propulsive function, whereas the octopus moves by its tentacles (Moral *et al.*, 2002).

Lipid

Lipid is a minor composition of cephalopod. The squid mantle contains only 1.0-2.0% (Okuzumi and Fujii, 2000). The lipid profile of cephalopod is clearly distinct from other species since it was rich in phospholipid. Triglyceride has been reported as a minor component of the flesh of cephalopods (Jangaard and Acman, 1965). Southgate and Lou (1995) reported that adult cephalopods are rich in long chain polyunsaturated fatty acid (PUFA). Their oil is a good source of

PUFA, which is often used in aquaculture to supplement feed. Lipid compositions of mantle meat in several squid species are shown in Table 2. Though varying from species to species, phospholipids account for about 38.1-84.0%.

Table 2. Lipid composition of squid mantle (%)

Name	PL	ST	FFA	TG	OT
Japanese common squid*	71.7	19.9	6.5	0.8	1.1
Spear squid*	83.8	14.8	1.1	0.3	ND
Neon flying squid*	61.8	15.8	21.6	0.1	0.7
Argetine shortfin squid*	73.1	15.4	6.8	3.2	1.5
Mako gonate squid*	84.0	3.6	3.6	trace	trace
Common cuttlefish**	39.7	ND	ND	1.42	ND
Loligo**	39.5	ND	ND	2.41	ND
Octopus**	38.1	ND	ND	2.51	ND

PL = phospholipid, ST = sterol, FFA= free fatty acid, TG = triglyceride,

ND = non-detectable, OT = steryl ester + unknown substances.

Source: Passi *et al.* (2002)*; Okuzumi and Fujii (2000)**

Navarro and Villanueva (2000) characterized lipid classes and fatty acid composition of three hatching species of cephalopod: *Sepia officinalis*, *Loligo vulgaris* and *Octopus valgaris*. All of 3 species contained high unsaturated fatty acid (62.7%, 61.4%, 60.1%), especially polyunsaturated fatty acid (PUFA). The n-3 fatty acids ranged from 18 to 25% of total lipid. In general, phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cholesterol (Chol) accounted for more than 60% of the total lipid. Squid and cuttlefish were richer in PC (34, 33%) and PE (25, 22%) as compared to newly hatched octopus. Saturated fatty acid contents of all 3 species were 27.2%, 35% and 35.7%

respectively. Palmitic acid was a main component of saturated fatty acid in cephalopod. *Sepia officinalis*, *Loligo valganis* and *Octopus valgaris* contained high proportions of C16:0 and C18:0. Oleic acid (C18:1 n-9) ranged from 12.1% to 19.1% in the triglyceride fraction and from 8.1 to 9.2% in the phospholipid fraction, whereas C22:6 n-3 and C20:5 n-3 ranged between 10.0% to 20.1% and 9.6 to 14.03% in the triglyceride fraction and between 24.5 to 35.9% and 7.07 to 21.2% in the phospholipid fraction, respectively (Passi *et al*., 2002). Takagi *et al*. (1986) reported that DHA and EPA are the most characteristic acids for cephalopods, ranging between 20 to 26% and 8.3 to 17.3% of total fatty acids, respectively. Arachidonic (C 20:n-4) was found in mantle between 1.1-1.9%. Culkin and Morris (1970) also reported that arachidonic acid constituted 1-2% of total fatty acid in cephalopod of the North Atlantic, while Gibson (1983) found arachidonic acid at a level of 1.8% of total fatty acid in octopus from Southern Australia.

Oxidative stability of lipids from squid tissues by comparing with those of other marine lipids was reported. Squid viscera or squid muscle with skin were most oxidatively stable, followed by squid eye total lipid (TL), trout egg TL, bonito oil and tuna orbital TL, respectively (Cho *et al*., 2001). Analyses of tocopherol contents and lipid compositions suggested that the higher oxidative stabilities of three kinds of squid tissue TL and trout egg TL compared to those of bonito oil and tuna orbital TL would be mainly because of the presence of phospholipids (PL) in squid tissue lipids and trout egg TL. However, the oxidative stability of lipids containing PL did not always decrease with increasing PL contents and stability was strongly influenced by PL composition (Cho *et al*., 2001). As squid viscera contained more than 25% of TL and these lipids were oxidative stable, squid viscera may be used as a good resource of functional lipids rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Passi *et al*., 2002).

Freezing and frozen storage

Freezing is the unit operation in which the temperature of food is reduced below the freezing point. A proportion of water undergoes a change to form ice crystals. The immobilization of water to ice, resulting in the concentrated dissolved solute in unfrozen water, lower the water activity of the food. Preservation is achieved by a combination of low temperatures and reduced water activity (Sikoski and Pan, 1994). During frozen storage of fish, the deterioration in quality due to microorganisms and some biochemical processes is decreased. Good quality of lean fish, which has been properly frozen and packaged, can normally be held at -20 to -30 °C for more than 1 year without the loss in consumer acceptability (Mackie *et al*., 1986). However, during frozen storage, physiochemical changes occurred, particularly those influencing lipids and proteins. This contributes to the loss in quality of fish muscle during frozen storage (MacDonal and Lanier, 1991; Haard, 1992).

Factors influencing deterioration during frozen storage include rate of freezing, temperature and time of frozen storage, fish species, post-harvest history of fish prior to frozen storage and cryoprotective compounds (Haard, 1992). Partial dehydration due to freezing of water and the associated concentration of solutes in the tissue mainly contributes to those changes (Figure 4) (MacDonal and Lanier, 1991). Frozen fish which has undergone deterioration during storage appear to accumulate high molecular weight protein aggregate stabilized by hydrophobic interaction as well as by disulfide bonds and other covalent cross-links (Haard, 1992) (Figure 4). These aggregates are initially become inextractable even in the presence of sodium dodecyl sulfate (SDS) and mercaptoethanol (ME) (Tejada *et al*., 1996). Formation of formaldehyde (FA) may also occur during frozen storage. Formaldehyde accelerates the formation of high-molecular-weight polymer from isolated

myosin and actomyosin during freezing and frozen storage (Aug and Hultin, 1989). Modification of relatively small number of amino acid side chain of associated myosin by FA is accompanied by major alterations in solubility and ATPase activity. By interacting with the side chain group on the fish protein, FA could increase the rate of protein denaturation during frozen storage. The denaturation would be mainly due to increased exposure of hydrophobic groups and this would lead to subsequent aggregation by non-covalent force (Haard, 1992). Myofibrillar proteins undergo denaturation and aggregation with decreased protein functionality, resulting in reduced water-binding capacity and changes in texture. The changes are extremely important for the subsequent technological treatment and they are associated with its deterioration.

When frozen cephalopods are stored, a series of significant changes occur. Ruiz-Capillus *et al.* (2002) studied the functional properties of voladur (*Illex coindentii*) muscle proteins during frozen storage at -20 °C for 16 months. Solubility of protein in 5% NaCl showed a significant increase in the initial months with subsequent decrease. This solubility was generally greater in the mantles than in the arms. The viscosity and emulsifying capacity values were initially very high and fell rapidly after 2 months. Soluble collagen in an acid medium decreased in both the mantle and arm throughout frozen storage. The greater change was also observed in arm than in mantle. However, myofibrillar and sarcoplasmic protein solubility of octopus (*Eledone arrhosa*) and pota (*Todarropsis eblane*) muscle in 5% NaCl remained over 60% throughout the frozen storage period of 12 months at -20 °C. The solubility of proteins from mantle of both pota and octopus in 5% NaCl was greater in young specimens than in mature sample. The acid solubility of collagen of pota and octopus also increased up to the sixth month. Pota collagen was significantly more soluble in mantles than in arms. In octopus mantle, collagen solubility gradually declined during frozen storage. For both immature and

mature stage, no gender-dependent differences were detected for the solubility of collagen from octopus mantle and arms. The mantles of immature specimen of pota and octopus were more suitable for freezing (Moral *et al*., 2002).
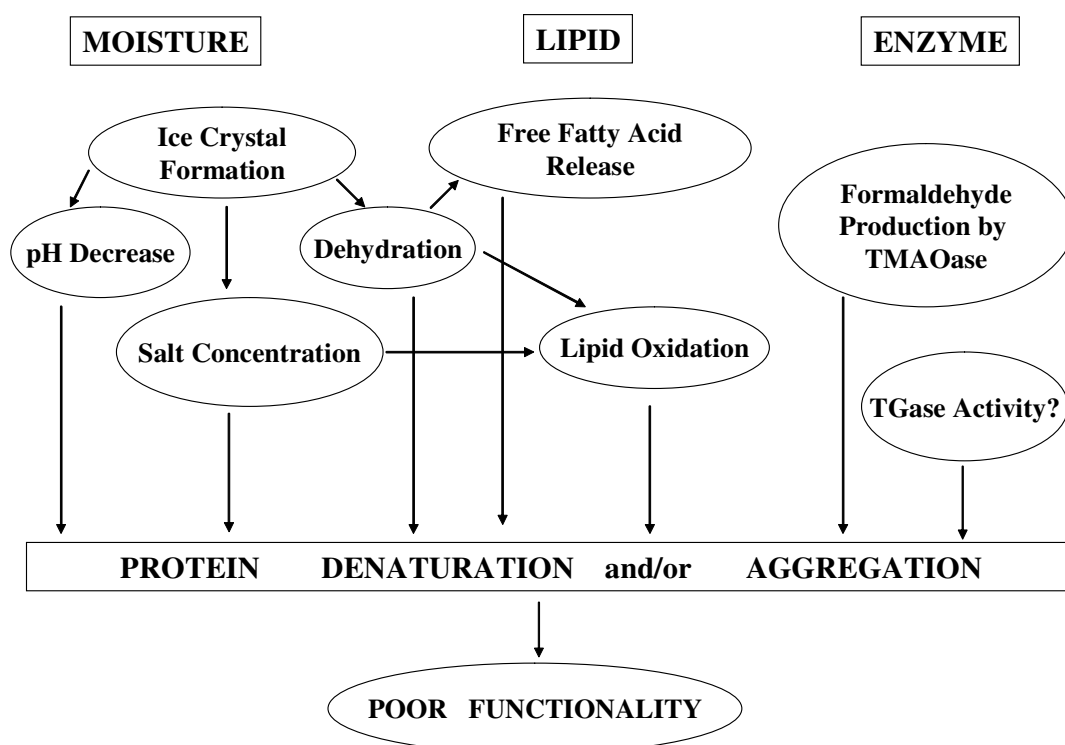

Figure 4. Factors affecting protein denaturation and aggregation during frozen storage.
Source: MacDonald and Lanier (1991)

Biochemical changes of actomyosin from mantles of squid (*Illex aryentinus*) at different sexual maturation stages took place during frozen storage (Paredi and Crupkin, 1997). The decreased extractability of actomyosin was accompanied by a simultaneous decrease in both viscosity and Mg^{2+}-ATPase activity. Myosin was more affected than other major myofibrillar proteins during frozen storage. These changes were independent of the sexual maturation stage of the specimens.

Irrespective of the sexual maturation stage of specimens, the extractability of actomyosin decreased by 50-55% during 3 months of frozen storage and decreased slowly thereafter. A gradual decrease in protein extractability during frozen storage of squid (*L. duvauceli*) was also reported (Joseph *et al*., 1977). However, extractable actomyosin from frozen squid (*O. Sloani pacificus*) decreased slightly even after a frozen storage and the long freezing period (Iguchi *et al*., 1981).

Frozen storage led to the reduced viscosity of actomyosin of squid mantle. Similar results were obtained with actomyosin from mantles of squids (Joseph *et al*., 1977). Mg^{2+}-ATPase activity in actomyosin from mantles of both immature and mature specimens decreased by 60% during 3 months of frozen storage. Iguchi *et al*. (1981) reported a slight decrease in the Ca^{2+}-ATPase activity of actomyosin extracted from frozen stored mantles of squid (*O. Sloani pacificus*). A decrease in band intensity of MHC and myosin light chain (MLCs) of actomyosin was observed during the frozen storage of squid mantles. The relative percentage of myosin significantly decreased and that of actin increased during frozen storage. Paramyosin increased slightly during 3 months of frozen storage and then decreased up to the end of storage (Iguchi *et al*., 1981). With the extended frozen storage, the texture of cephalopod became though and the acceptability in lowered. Ueng and Chow (1998) found the toughness of three species of cephalopods (*Loligo edulis*, *Sepia pharaonis* and *Illex argentinus*) increased during frozen storage at -20 °C for 4 months. There was no significant difference in drip and SDS gel electrophoresis patterns after 4 months of frozen storage among all species.

Microstructure observation of mantle showed that the muscle fibers were injured and aggregated while the frozen time increased. Those changes in tissue structure during frozen storage might cause the toughening of mantle. Stanley and Hultin (1982) reported that the toughening of frozen squid

mantle might be caused by protein cross-linking because of the existence of high levels of DMA and formaldehyde. The frozen mantles were tougher than the unfrozen, and the longer-period frozen-stored mantles were tougher than the shorter-period frozen-stored. The formation and growth of ice crystals might have injured the muscle fiber and enhanced the protein aggregation that caused toughening of the mantle (Ueng and Chow, 1989).

Lipid oxidation and its effect on protein denaturation and discoloration of muscle food during processing and storage

Lipid oxidation in muscle foods is one of the major deteriorative reactions causing the losses in quality during processing and storage. The overall mechanism of lipid oxidation and its effect on the changes of quality in food during processing and storage are shown in Figure 5. The oxidation of unsaturated fatty acids or triglyceride leads to formation of free radicals and hydroperoxides. Such intermediary compounds are unstable and cause oxidation of pigments, flavors, and vitamins. After polymerization, hydroperoxides form dark colored organic polymers (Shoo and Verma, 1999). Other compounds such as ketones, aldehydes, alcohols, hydrocarbons, acids, and epoxides are formed during oxidation of unsaturated fatty acids (Khayat and Schwall, 1983). The lipid oxidation processes lead to discoloration, drip losses, off-odor and off-flavor development and production of potentially toxic compounds (Morrissey *et al*., 1998). Oxidized unsaturated lipids bind to protein and form insoluble lipid-protein complexes. This accounts for the toughened texture, poor flavor and unappealing odor of poorly stored frozen seafood (Khayat and Schwell, 1983). Deterioration of commercial channel catfish can occur within 4 months of storage (-18 °C) and results in undesirable changes in the tissue, including development of rancid off-flavor, changes in texture, color, water-holding capacity, and nutritive properties, and

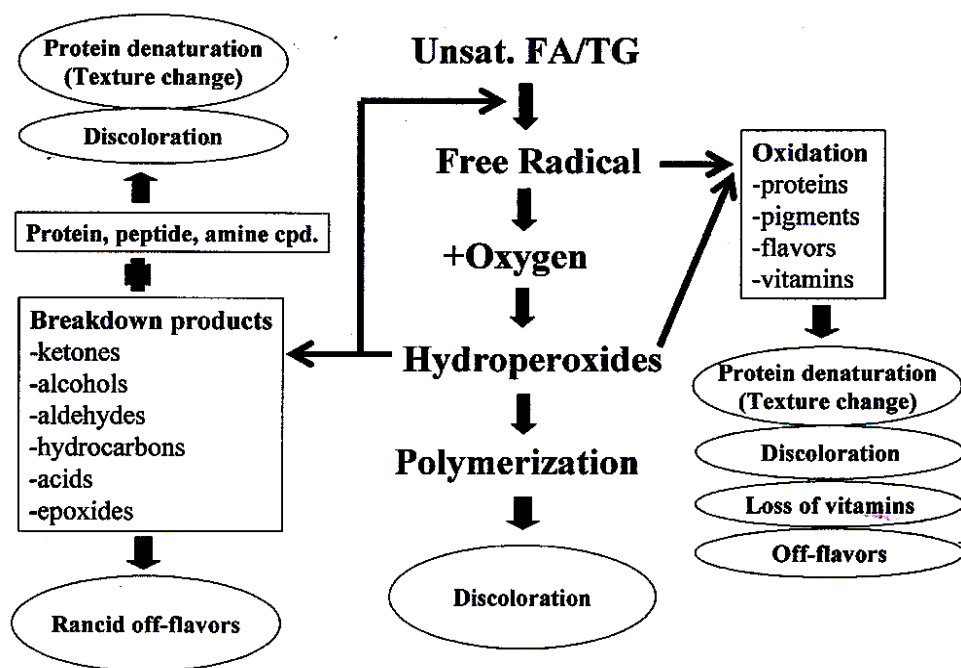increased safety risks associated with oxidized products (Thed *et al.*, 1993).

Figure 5. The overall mechanism of lipid oxidation and its effect on the changes of quality in food during processing and storage.

Source: Jadhav *et al.* (1996)

Mechanism of lipid oxidation

The classical mechanism for lipid oxidation is via free radical attack and initiation substrate for lipid oxidation unsaturated lipids. The free-radical chain mechanism has been generally accepted as the only process involved in autoxidation. The following simplified scheme shows the various steps in autoxidation (Khayat and Schwall, 1983):
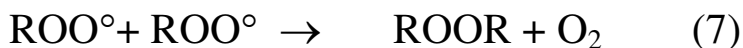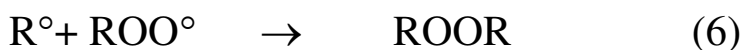
A. Initiation step

$$RH \rightarrow R° + H° \quad (1)$$
$$RH + O_2 \rightarrow ROO° + H° \quad (2)$$

B. Propagation step

$$R° + {}^3O_2 \rightarrow ROO° \quad (3)$$
$$ROO° + RH \rightarrow ROOH + R° \quad (4)$$

C. Termination step

$$R° + R° \rightarrow R\text{-}R \quad (5)$$
$$R° + ROO° \rightarrow ROOR \quad (6)$$
$$ROO° + ROO° \rightarrow ROOR + O_2 \quad (7)$$

where RH is unsaturated lipid, R° is a lipid radical, ROO° is a lipid peroxy radical and ROOH is hydroperoxide. At initiation step, free radicals are formed when loosely held hydrogen atom is abstracted from "active methylene" located between two double bonds. Initiators of this step include trace metal, light of heat (Eq. 1, 2). The fat free radicals react with oxygen to form peroxy free radicals. These peroxy free radicals act as strong initiators or catalysts of further oxidation by extracting a hydrogen atom from another molecule, triggering propagation (Frankel, 1984). During the propagation step, the peroxy radicals remove a hydrogen atom from a lipid to form a relatively stable hydroperoxides (ROOH) and a new unstable fatty radical (Eq. 3, 4). Lipid hydroperoxides, the primary products of autoxidation, are odorless and tasteless. The unstable fatty radical will then react with oxygen to form
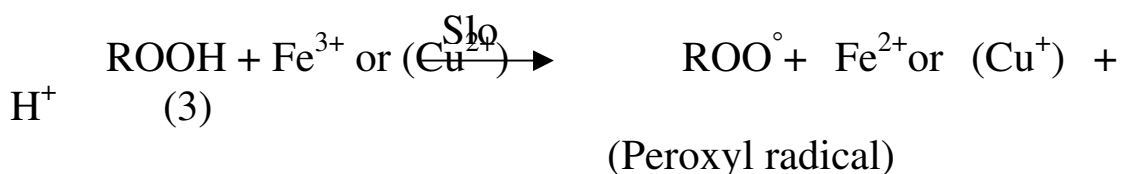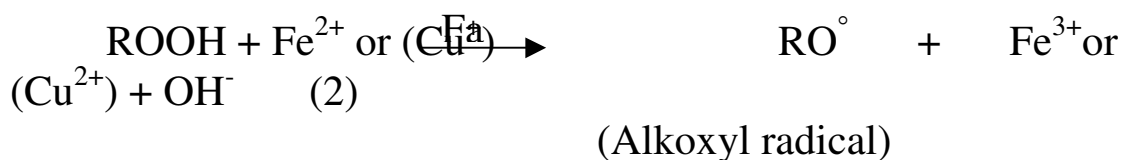
another new reactive peroxy radical. This reaction, when repeated many times, produces an accumulation of hydroperoxides. The propagation reaction becomes a continuous process as long as unsaturated lipid or fatty acid molecules are available (Frankel, 1984). For termination, the hydroperoxides split into smaller short chain organic compounds e.g. aldehydes, ketones, alcohols and acids, which cause the off-odors and off-flavors characteristic of rancid fats and oils. The auto-oxidative process is ended when two unstable radicals react with each other (Eq. 5, 6, 7) (Khayat and Schwall, 1983).

The important factors affecting lipid oxidation

Fatty acid composition: Type of fatty acids, degree of unsaturation and proportion of phospholipids are the important factor affecting lipid oxidation. Hydrogen abstraction occurs much easier in unsaturated fatty acids than in their saturated counterparts. The number, position and geometry of double bonds affect the rate of oxidation. Cis acids are oxidized more readily than their trans isomers and conjugated double bonds are more reactive than non conjugated (Nawar, 1996). The number of double bonds in a fatty acid affects its susceptibility to oxidation; the higher the number of double bonds, the more prone to rancidity. Addition of each of the unsaturated fatty acids: palmitoleic acid (16:1, n-7), linoleic acid (C18:2, n-6), eicosapentaenoic acid (EPA: C20:5, n-3) and docosahexaenoic acid (DHA; C22:6, n-3) to fresh minced salmon changed the sensory perception and increased the intensity of bitterness and metal taste in the following order: DHA>palmitoleic acid>linoleic acid>EPA (Refsgaard *et al*.,

2000). Relative rates of oxidation for arachidonic, linolenic, linoleic, and oleic acid are approximately 40:20:10:1, respectively (Nawar, 1996).

Metal: Transition metal ions are remarkable good promoters of free-radical reaction because of singlet electron transfer during their change in oxidation states. A direct reaction between a metal catalyst and a lipid molecule is envisaged in the chain initiation step (1). Nonenzymatic lipid oxidation is enhanced by metal ions like iron, cobalt, and copper, as well as heme compounds (2), (3) (Jadhav *et al.*, 1996). The reduced state of copper and iron are capable of promoting lipid oxidation by catalyzing the decomposition of hydrogen peroxide and lipid peroxides to free radicals such as the hydroxyl radical (Kanner *et al.*, 1990). About two-thirds of body iron is found in hemoglobin and smaller amounts in myoglobin. Iron, ascorbate and activated metmyoglobin generated different oxidative responses in chicken muscle model systems (Sista *et al.*, 2000). In iron ascorbate systems, large increases in hydroperoxides and thiobarbituric acid reactive substances (TBARS) occurred during the initial stage of incubation. Thereafter, iron-ascorbate catalysis led to a slow increase in the oxidation of triacylglycerol and sarcoplasmic reticulum membrane lipids. In addition, Fe^{3+} can be reduced to Fe^{2+} by reductants such as ascorbic acid and compounds containing thiol groups (Dunford, 1987), thereby increasing iron-induced lipid oxidation.

$$Mn^{2+} + R\text{-}H \longrightarrow Mn^{+} + H^{+} + R^{\circ} \qquad (1)$$

$$ROOH + Fe^{2+} \text{ or } (Cu^{+}) \xrightarrow{Fast} RO^{\circ} + Fe^{3+} \text{ or } (Cu^{2+}) + OH^{-} \qquad (2)$$

(Alkoxyl radical)

$$ROOH + Fe^{3+} \text{ or } (Cu^{2+}) \xrightarrow{Slow} ROO^{\circ} + Fe^{2+} \text{ or } (Cu^{+}) + H^{+} \qquad (3)$$

(Peroxyl radical)

Temperature: The rate of oxidation increases with increasing temperature. The high temperature can cause many isomerization and scission reaction to take place, producing a myriad of secondary or breakdown products such as epoxies, dihydroperoxides, cyclized fatty acids and dimers. With scission reactions, aldehydes and ketones are found (Jadhav *et al*., 1996). Aubourg (1999) reported that blue whiting fillets frozen at -40 °C, -30 °C and -10 °C up to 1 year underwent primary and secondary lipid oxidation especially at -10 °C of storage, while at -30 °C of storage, a significant increase in free fatty acid content was obtained at 5 months and then no more increases were detected. Saeed and Howell (2002) studied the effect of lipid oxidation and frozen storage at -20 °C and -30 °C for 24 months on muscle proteins of Atlantic mackerel. An increase in lipid oxidation products was obtained with increasing storage time and at a higher storage temperature. Antioxidants had a significant effect on the inhibition of lipid oxidation, as shown by the reduction in peroxide value and hydroperoxides, and malondialdehyde formation.

Oxygen and surface area: The rate of oxidation increases in direct proportion to the surface area of the lipid exposed to air. As surface-volume ratio is increased, a given reduction in oxygen partial pressure becomes less effective in decreasing the rate of oxidation (Nawar, 1996). Non-enzymatic and enzymatic oxidations of fatty acids appear to require conversion of ground state (triplet oxygen) to singlet oxygen and superoxide dismutase or singlet oxygen quenchers can prevent such oxidation (Khayat and Schwall, 1983).

Water activity: The rate of oxidation depends strongly on water activity. Foods with very low moisture content (a_w value of less than about 0.1) undergo oxidation very rapidly. Increasing the a_w to about 0.3 retards lipid oxidation and often produces a minimum rate. This protective effect of small

amount of water is believed to occur by reducing the catalytic activity of metal catalysis, by quenching free radicals, and/or by impeding access of oxygen to the lipid. At somewhat higher water activities (a_w=0.55-0.85), the rate of oxidation increases again, presumably as a result of increased mobilization of catalysts and oxygen (Nawar, 1996).

Effect of lipid oxidation on protein denaturation

Muscle tissue contains high concentration of oxidizable lipids, heme pigments, transition metal ions, and various enzymes. These substances serve either as precursors or catalysts for the production of reactive oxygen species (ROS) and non oxygen free radicals (NOR), non radical species such as H_2O_2, ROOH, and various reactive aldehyde and ketones. Those radical and compounds contribute to the changes of chemical, physical, and functional properties of proteins in meat, particularly via oxidation process (Kanner, 1994). In the presence of oxidized lipids, protein oxidation is manifested by free radical chain reactions similar to those for lipid oxidation, which involve initiation, propagation, and termination (Gardner, 1979). Oxidation reactions can lead to the formation of protein radicals, polymers and protein-lipid complex. The sites of free radical attack on protein include both the amino acid side chains and the peptide backbone. The attack often results in either protein polymerization or fragmentation (Xiong, 2000).

Reactive oxygen species (ROS) mediated formation of protein-protein cross-linked derivatives in meat can occur by the following mechanisms (Xiong, 2000).

1. By the oxidation of cysteine sulfhydryl groups to form disulfide linkages.

2. By the complexing of two oxidized tyrosine residues.

3. By the interaction of an aldehyde group in one protein with the ε-NH_2 group of a lysine residue in another protein.

4. By the cross-linking of two ε-NH$_2$ groups (lysine residues) in two proteins through a dialdehyde (a bifunctional agent, e.g. malondialdehyde and dehydroascorbate).
5. By the condensation reaction of protein free radical.

Physicochemical changes of protein

Degradation and polymerization of myofibrillar proteins were accelerated in presence of different model oxidation systems, closely resembling meat or processed meat condition (Decker *et al*., 1993; Martinard *et al*., 1997; Srinivasan and Hultin, 1997; Liu *et al*., 2000; Liu and Xing, 2000). Liu and Xiong (2000) found the marked changes in electrophoretic pattern of myosin treated with FeCl$_3$/H$_2$O$_2$/ascorbate system. Oxidation caused disulfide cross-linkage of myosin to form polymer. The myosin molecule has ~42 sulfhydryl groups, which can be readily accessed by chemical reagents. Upon exposure to hydroxyl radicals, the sulfhydryl groups of MHC would be oxidized to inter-molecular disulfide bonds. Thus, oxidation not only caused fragmentation of MHC but also cross-linking of MHC, producing oligomer or polymers (Liu and Xiong, 2000).

The structural integrity and conformational stability are afforded by the specific intramolecular forces as well as the interactions between the surface amino acid and the surrounding water molecules. The various intramolecular and intermolecular forces minimize the free energy of whole system (Kanner, 1994). When subjected to ROS attack, the original physical and chemical forces that stabilize the proteins are disrupted, and this can result in decreased thermal stability of the protein molecules (Xiong, 2000). Myosin is highly susceptible to free radical attack. An exposure to °OH produced from a free radical-

generating system [Fe^{3+}/ascorbate/H_2O_2] resulted in a major reduction in the thermal stability of myosin (Liu and Xiong, 2000). In addition to causing protein fragmentation and polymerization, °OH is also capable of modifying side-chain groups of many amino acid residues. Certain amino acid residues, for example, histidine, lysine, and arginine are particularly sensitive to °OH oxidation (Amici *et al*., 1989). Disturbance of ordered protein structure can be measured by determination of the UV absorbance of protein solution. Myofibrillar protein exposed to oxidative systems (iron, copper and linoleic acid in the presence of ascorbate) exhibits increased absorption within the 250-290 nm wavelength range (Decker *et al*., 1993). The UV absorbance of oxidized protein increased with increasing ascorbate concentration up to 25 mM. Meucci *et al*. (1991) also reported enhanced light absorption of human serum albumin oxidized by 100 mM ascorbate and trace minerals.

Denaturation resulting from unfolding of oxidized muscle protein has also been analyzed using the hydrophobicity measurement. Food proteins in their native structure have a similar amino acid distribution. The polar (hydrophilic) residues generally exposes to water while the nonpolar (hydrophobic) groups or moieties generally reside in the molecule (Amici *et al*., 1989). Wang *et al*. (1997) reported major increases in surface hydrophobicity of beef heart surimi protein stored at either -15 or -29 °C for more than three months. The enhanced hydrophobicity closely paralleled to the oxidation of lipids and the disappearance of free sulfhydryl groups. Under the freezing stress condition, protein structural changes may result from oxidative modification of amino acid residue side chains that are presumably initiated by lipid free radical. Denaturation of protein structure and functionality in mackerel stored for 3, 6, 12 and 24 months was greater at -20 than -30 °C. ATPase activity in the myosin extract of Atlantic mackerel showed a

significant decrease with progressive frozen storage. Protein solubility in high salt concentration (0.6 M NaCl) decreased during storage at both -20 and -30 °C, but it was greater at -20 °C (Saeed and Howell, 2002). This was probably associated with higher oxidation at higher temperature. Li and King (1999) studied interaction between rabbit myosin subfragment with malonaldehyde, a byproduct of lipid oxidation in a model system containing the subfragment 1 and malonaldehyde. Malonaldehyde caused cross-linking or polymerization of the protein. Malonaldehyde also reduced α-helix content, increased random structure, and eliminated some β-strand structure in subfragment 1. Tunhun *et al.* (2000) reported that washing carp surimi with $CuCl_2$ solution resulted in a decrease in the total SH content of fish meat and cross-liking of myosin heavy chain (MHC) through disulfide bonding which resulted in the weakening of its gel forming ability. The addition of 10% of oxidized cod liver oil caused the polymerization of myosin heavy chain of Alaska Pollack surimi gel during setting at 30 °C resulted in a decrease of breaking force of surimi gel (Murakawa *et al.*, 2003). Oxidation of natural actomyosin (NAM) of bigeye snapper with the free radical-generating system resulted in an increase in protein carbonyl content. SDS-PAGE pattern showed that the aggregation of oxidized NAM was not caused by disulfide bond. Therefore, the aggregation of NAM may be formed by Schiff base adducts, or the formation of carbon-carbon covalent bonds (Visessanguan *et al.*, 2003)

Changes in functional properties of protein

Myofibrillar proteins, especially myosin, are largely accountable for the functional attributes of muscle food. The exact functionality changes, which can be beneficial or detrimental, depend on the oxidative processes and conditions

such as type of the concentration of oxidants, length of oxidation, etc. (Xiong, 2000). Hence, characterization of the functional properties of myosin under oxidative stresses would provide insight into the mechanism of changes that occur to the myofibrillar protein mixture. Decker *et al.* (1993) reported that hydroxyl radicals promoted the deterioration in functional properties of myofibrillar proteins (solubility, water holding capacity and gel strength). Oxidative reactions catalyzed by both iron and copper (25 μM) in presence of ascorbate (10 mM) severely weakened the gel-forming ability of turkey myofibrils as manifested by weakening of the gel matrix structure. Because of their porosity, gel made from the oxidized protein was able to hold only 33-73% as much water as gels made from non-oxidized protein. The losses in the functional properties correlated with decreased in protein solubility and structural stability and with increased carbonyl groups. Those changes resulted from excessive cross-linking of proteins through carbonyl-amine reactions (Xiong, 2000). Between two prooxidative metal ions, iron caused greater changes in protein conformation, generation of carbonyl derivatives, formation of protein polymers, and loss of myosin and actin solubility, although it was largely similar to copper in affecting the gelation of the protein. This could be due to the fact that iron is more effective than copper in catalyzing the formation of hydroxyl free radicals (Decker *et al.*, 1993). A decrease in protein solubility is the result of a shift from a balance of protein intramolecular interaction and protein-water interaction to a situation where protein intermolecular interaction is favored while protein-water interaction is weakened. Hence, in addition to the increase in surface hydrophobicity as a result of loss of ordered tertiary structure, cross-linkages formed among proteins undergoing free radical attack are also a major cause of decreased protein solubility (Xiong, 2000). Forces involved in the cross-linking of oxidized proteins include both covalent and noncovalent bonds. In particular, protein-protein interactions via

the bifunctional malondialdehyde have been found to be a major cause for protein solubility decrease in meat and meat products (Buttkus, 1967; Dillard and Tappel, 1973).

Liu and Xiong (1996) showed that myofibrils isolated under different antioxidative condition from three groups of chicken muscle [breast (Bctrl), leg (L) and breast with its iron and fat content adjusted to the level of leg muscle (Badj)] exhibited different viscoelastic characteristics during thermal gelation. For non antioxidant condition, Badj myofibrils had a decreased storage modulus (G') when compared with Bctrl myofibrils, but these values were much higher than those of L myofibrils. This study has shown that the poultry dark (red) and light (white) muscle differ in functionality under oxidative condition. Red muscle contains not only a large amount of lipids but also a high concentration of heme protein and inorganic iron, both of which are catalysts for lipid and protein oxidation (Monahan et al., 1993). It is also possible that there are specific iron-binding sites on myofibrillar proteins, which allow more efficient transfer of metal-generated free radicals to the proteins. Considering its greater potency and higher concentration in muscle tissue, iron is more important catalyst of protein oxidation than copper in muscle food (Decker et al., 1993). Liu et al. (2000) found that myofibrils oxidized by $FeCl_3/H_2O_2$/ascorbate had the decrease in gel-forming ability. The decrease in viscoelastic pattern (storage modulus: G') of oxidized myofibrils might result from alteration in the functional groups of myofibrillar proteins. Moreover, it could be due to disulfide bond formation. While the formation of disulfide cross-links is beneficial for strengthening the gel network during thermal gelation, excessive cross-linkage of myofibrillar protein before heating would generate large protein aggregates, which might hinder ordered interaction of reactive functional groups and inhibit formation of a fine gel network (Liu et al., 2000).

Changes in color

Lipid oxidation products are involved in browning processes by reacting with primary and secondary amino groups of proteins or amino containing compounds such as phospholipids. The role of phospholipids, or other lipids, in nonenzymatic browning reaction is consequence of their ability to be oxidized (Hidalgo *et al*., 2005). Particularly, the reaction of lipid oxidation products with amino group of amines, amino acid and protein is associated with the browning observed in many fatty foods during processing and storage (Pokorny and Kolakowska, 2002). The reaction of lipid oxidation products with primary amino groups of phosphatidylethanolamine or phosphatidylserine also takes place. Pyrroles formed in the reaction of oxidized lipids with protein are important precursors of both brown and fluorescing compounds (Zamora *et al*., 2000). Browning reaction was observed that produced by intermolecular or intramolecular interaction between amine group of phospholipids and hydroperoxides or their carbonylic degradation products. Pyrroles are common products in the reaction of different lipid oxidation products with the primary amino groups of amines, amino acids, and proteins (Figure 6). The resulting products of oxidized lipids are yellow, red or brown in color (Pokorny, 1974). The color intensity increases rapidly with increasing unsaturation of the original lipid fraction, and correlates with loss of primary amine groups (Pokorny, 1981). The character of brown products is similar to that of melanoidines from Maillard reaction (Hidalgo *et al*., 1993).

Yellow/brown discoloration is associated with lipid oxidation in some kinds of muscle foods such as fish (Lauritzen and Martinsen, 1999; Hamre *et al*., 2003) and turkey (Yu *et al*., 2002). Lauritzen and Martinsen (1999) studied copper induced lipid oxidation during salting of cod. The yellow/brown color was relatively evenly distributed in all section of cod fillets.

However, the ventral part of the fillets was more prone to discoloration, compared to the others. External factors such as contaminants present during the processing may be the major cause of this problem. The amount of copper correlated with yellow color and lipid oxidation of the muscle blends. The copper concentration increased from approximately 1 ppm to 5 ppm with the increase in yellow color of the samples. A positive correlation between the copper content, lipid oxidation (TBARS) and yellow color is clearly demonstrated. During frozen storage of the Norwegian spring-spawning herring up to 9 weeks, there was an increase in lipid oxidation products as evidenced by the increase in TBARS and PV. The concomitant loss of red color and the substantial increase in yellow color caused a visible change in appearance (Hamre *et al.*, 2003). Yu *et al.* (2002) studied lipid oxidation and color change in cooked turkey products during refrigerated storage. The increase of lipid oxidation was correlated with the decrease of redness value (a*-value) and the increase of yellowness value (b*-value). Nevertheless, lipid oxidation and discoloration of turkey products were protected by rosemary extract.

Pokorny *et al.* (1974) studied the discoloration mechanism of fish muscle in model systems consisting of methylesters of polyunsaturated fish oil fatty acid and fish muscle (cod, carp, and mackerel) homogenates, fish myosin, or pure protein of animal origin. Both soluble and insoluble brown pigments and flavor substances resembling fishy order were produced by interaction of lipid peroxide and carboxylic peroxide decomposition products with primary and secondary amino groups of protein. Browning reactions of lipid-protein mixtures proceeded by autoxidative reactions of lipid phase and the formation of brown pigments was prevented by addition of antioxidant (El-Zeany *et al.*, 1975). The pigments are probably formed by ionic condensation of primary amino groups of protein with conjugated unsaturated aldehydes or similar active lipid-oxidation products, produced by cleavage of unsaturated

hydroperoxides (Kimoto and Gaddi, 1971). The intermediary Schiff bases are converted into high-molecular-weight brown pigments by subsequent aldolization reaction. These processes cause the deterioration of food during storage and processing and contribute to destruction of essential amino acids, decrease in digestibility, inhibition of proteolytic and glycolytic enzymes, interaction with metal ions, and formation of antinutritional and toxic compounds (Friedman and Gumbmann, 1986).
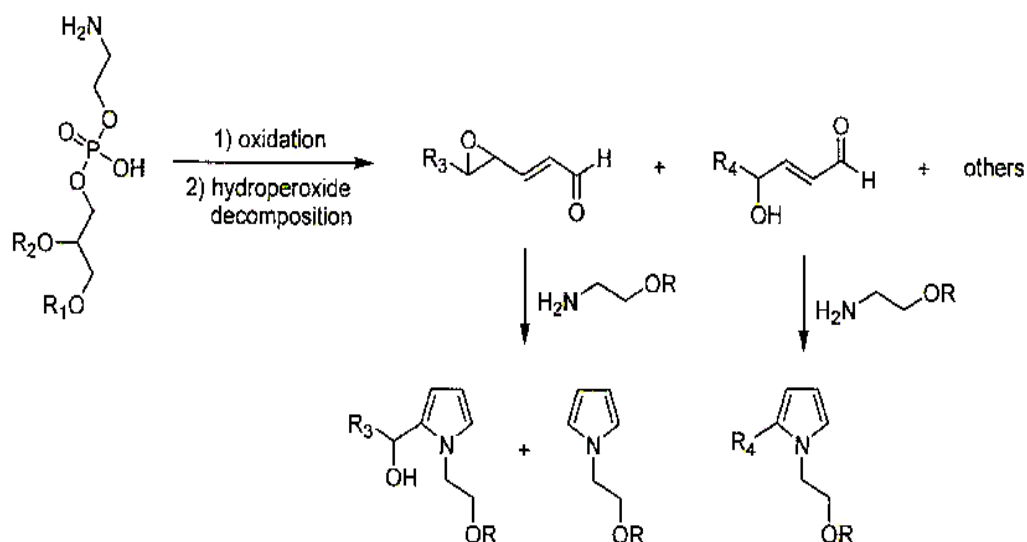

Figure 6. Production of reactive carbonyls during phosphatidylethanolamine oxidation and the later formation of pyrrolized phospholipids by amino-carbonyl reactions. R is the phospholipid without the polar head; R1 and R2 are fatty acid chains; and R3 and R4 are alkyl chains.

Source: Hidalgo *et al.* (2005)

Lipid oxidation of microsome and liposome systems

Microsomes are the small particles in the cytoplasm of a cell, typically consisting of fragmented endoplasmic reticulum to which ribosomes are attached. They are any of a number of minute granules in the cytoplasm of an active cell, thought to be associated with protein synthesis (Linberg and Ernster, 1954). Microsomes contain lipid about 43% of total dry weight, which mostly are the phospholipids membrane. Microsomes contain a large amount of oxidative enzymes (Linberg and Ernster, 1954). Membrane phospholipids have a high content of highly polyunsaturated fatty acids. The membrane phospholipids exist primarily in the form of a bilayer, in which a much larger surface area is exposed per unit weight of lipid. They exist primarily as inter- and intracellular fat droplets (Huang *et al.,* 1993). It had been observed in the 1960s that the microsomal fraction from liver had the capacity to catalyze lipid oxidation in the presence of NADPH and iron and this reaction was enhanced in the presence of ADP (Hochstein and Ernster, 1963). The presence of an enzyme-catalyzed lipid peroxidation system in the microsomal fraction of muscle tissue was demonstrated by Lin and Hultin (1976). Skeletal muscle contains high concentrations of unsaturated fatty acids, especially in cellular membranes such as mitochondria, sarcoplasmic reticulum and microsomes (Chan and Decker, 1994). Muscle microsomes and sarcoplasmic reticulum enzymically catalyze lipid oxidation in the presence of iron, ADP and NADH or NADPH (Lin and Hultin, 1976; Borhan *et al.,* 1984; and Rhee, 1988). Nonenzymic metal-catalyzed lipid oxidation occurs in the presence of reducing agents such as superoxide, ascorbate or cysteine (Kanner *et al.,* 1987). A similar lipid peroxidation system in the microsomal fraction of fish muscle was reported (McDonal *et al.,* 1979). Unlike the mammalian and avian muscle systems (Lin and Hultin, 1976; Rhee *et al.,* 1984), the microsomes of fish muscle functioned very much better in the presence of NADH, compared to

NADPH. In addition, the fish microsomal fraction was very active at relatively low temperature and even had significant activity in the frozen state (Apgar and Hultin, 1982).

McDonald and Hultin (1987) showed the proposed pathway of the enzymatic lipid oxidation in microsomal fraction of flounder muscle (Figure 7). The main suggestion of the reaction scheme suggests the role of the enzyme in maintaining iron in the reduced state. Some of reduced irons react with molecular oxygen to form an intermediate, indicated as the $Fe^{+2}O_2$, dioxygen ferrous in complex, in equilibrium with $Fe^{+3}O^{2-}$, preferryl iron. This released superoxide and Fe^{3+} forms a complex with ADP, whose function is to keep Fe^{3+} soluble and/or modify its oxidation-reduction potential. The function of enzyme (ENZ) is to reduce the Fe^{3+} to Fe^{2+} using electron from NADH. This reaction of iron is suggested to be the primary function of the membrane enzyme and occurs in two places in the scheme, indicated by enzyme. Superoxide is dismutated to O_2 and H_2O_2 which can then further react with O^{2-} to produce the hydroxyl free radical (^oOH) via a Fenton-type reaction. Superoxide dismutase inhibits the reaction by removing superoxides to prevent its reaction with H_2O_2 formed while the inhibitory effect of catalase is due to its ability to remove H_2O_2. The hydroxyl free radical may then abstract hydrogen from lipid to initiate lipid oxidation, which then proceeds through the usual process to form a hydroperoxide. The hydrogen peroxide can be decomposed by ferrous iron or ADP-complexed Fe^{2+} to produce a variety of free radicals and singlet oxygen. The effectiveness of the singlet oxygen scavenger, diphenylfuran, indicates that initiation of the reaction can also be accomplished by singlet oxygen. Cysteine in the presence of Fe^{3+} can also decompose lipid hydroperoxides to produces free radicals. These products may then be involved with further propagation reaction by interacting with other molecules of lipid to maintain of the chain reaction (McDonald and Hultin, 1987).

NAD$^+$ ENZ NADH ADP-Fe^{+3}

ADP+Fe^{+2} Fe^{+3}O$_2^{-o}$ Fe^{+3}

O$_2^-$ Fe^{+2}O$_2$

O$_2^{-o}$

NAD$^+$

ADP Fe^{+2} H$_2$O$_2$

NADH ENZ

ADP-Fe^{+3} Fe^{+3} O$_2^{-o}$

ADP

^{o}OH LH

L^o

ADP

NAD$^+$

CYS LOOH Fe^{+2} ENZ NADH

Fe^{+3}

OX.CYS Fe^{+3}+ADP ADP-Fe^{+3}

LO^o, LOO^o, O$_2^{-o}$, ^{o}OH, ^{1}O$_2$

END-PRODUCTS

Figure 7. A proposed pathway of the enzymatic lipid oxidation in microsomal fraction of flounder muscle.
Source: McDonal and Hultin (1987)

Liposomes are microscopic spherical vesicles that form when phospholipids are hydrated. When mixed in water under low shear conditions, the phospholipids arrange themselves in sheets. These sheets then join tails-to-tails to form a bilayer membrane, which encloses some of the water in a phospholipid sphere (Figure 8). Liposomes are useful model for biological membranes research (Kozubek *et al*., 2000). More recently, liposomes have been evaluated as delivery systems for drugs, vitamins and cosmetic materials. Liposomes have been used extensively as model to study lipid oxidation of membrane lipid. Yin and Faustman (1993) used the liposome system to study influence of the temperature, pH, and phospholipids composition upon the stability of myoglobin and phospholipids. Antioxidant effect of porcine plasma was studied in phosphatidylcholine liposome system (Faraji and Decker, 1991). Decker and Hultin (1993) used liposome model to study the factors influencing catalysis of lipid oxidation by soluble fraction of mackerel muscle. The inhibitory effect of phenolic

compounds from berry on protein and lipid oxidation was also studied in liposomes (Viljanen *et al*., 2004)

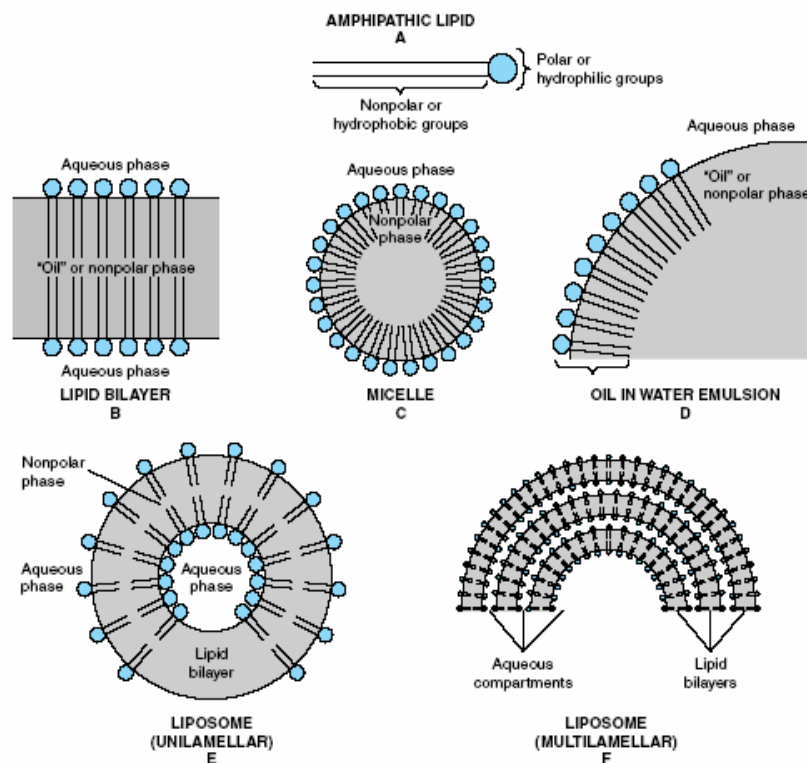**Fig** 　　　　　　　　　　　　　　　　　　　　　　　　　ions and liposomes from amphipathic lipids.
Source: Meyes and Kathleen (2003)

1.3 Objectives of study

1. To determine the chemical composition and some properties of cuttlefish (*Sepia pharaonis*) head and mantle.
2. To study the effects of metal ions on lipid oxidation, discoloration and physicochemical changes of cuttlefish muscle as affected by multiple freeze-thaw cycles.
3. To investigate the effect of lipid oxidation on the formation of yellow pigments in a cuttlefish phospholipid liposome system.
4. To study both enzymatic and nonenzymatic pathways of lipid oxidation in microsomal fraction of squid (*Loligo peali*) muscle.

5.   To elucidate yellow pigment formation both in squid protein and lipid fractions.

6. To investigate the effect of antioxidant on lipid oxidation, discoloration, protein denaturation and functional properties of cuttlefish muscle during frozen storage.