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Functional Properties of Fish Proteins : A Review

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Proteins possess a number of physiochemical characteristics which facilitates their varied food applications. Proteins exhibit a wide range of functionality and versatility during processing. Functional properties of proteins are mainly due to its interaction with other components within the food system. The structure of protein is highly dependent upon the environment and protein assumes different conformations depending upon the conditions of exposure. The surface hydrophobicity plays a major role in the functional properties of a protein and changes in the inherent characteristics influence protein structure which have profound influence on the functional properties of the protein.

Key words : Proteins, Functional properties, surface hydrophobicity

Proteins are endowed with a number of physiochemical characteristics, which make them suitable for varied food applications. Cells synthesize proteins by transcription from RNA sequences. When proteins are transcribed they start out as linear sequences of amino acids. Because these amino acids have varied electrostatic and mechanical properties, they fold up into a three-dimensional structure, which gives the protein its functionality, both physiologically and in food applications. The intrinsic properties like amino acid composition, amino acid sequence, conformation, size, charge and intermolecular bonding are some of the important factors that determine the functional properties of a protein (Kinsella, 1976; Damodaran, 1994). The total hydrophobicity of protein molecule also plays a crucial role in determining the functional properties. (Davis *et al.*, 1973; Horiuchi & Fukushima, 1978; Shimada & Matsushita, 1980).

Proteins exhibit a wide range of functionality and versatility during processing. Protein manifest these functionality by interacting with other components within the food involving solvent molecules, other protein molecules or substances that are dispersed in the solvent such as oil or air. Generally, proteins exist in the lowest kinetically attainable state of free energy. The structure of protein and its different conformations depend upon the environmental conditions namely pH, temperature, dielectric constant, ionic strength, presence of other molecules including air, fat, denaturants, etc. Proteins lower the free energy through removal of hydrophobic groups from its surface. The hydrophobic bonds are very sensitive to changes in temperature and dielectric constant and therefore changes in these parameters influence protein structure.

Fish proteins are unique in nature and exhibit high degree of functional properties. Functional properties can be defined as those physico-chemical properties, which affect the processing and behavior of proteins in the food system as judged by the final quality of the product (Kinsella, 1981). Based on solubility, fish proteins are classified as myofibrillar proteins (65-75%), sarcoplasmic or enzymic proteins (20-30%) and stroma or connective tissue proteins (1-3%). The functional and textural characteristics of meat depend mainly on myofibrillar protein, (Goll *et al.*, 1977) the protein associated with the muscle structure. For successful utilization of fish proteins in food applications, protein should ideally possess certain functional properties, which in turn is related to the composition and structure of protein and

their interaction with other substances present in the food (Colmenero & Borderias, 1983).

Protein solubility

Solubility is considered important for a protein to exhibit its functional properties (Kinsella, 1984; Kinsella & Whitehead, 1989). It is the quantity of protein that goes in to solution under specified conditions (Xiong, 1997) and is influenced by amino acid composition and sequence, conformation and the content of polar and non polar groups of amino acids in the protein (Zayas, 1997). The 3-D configuration of protein is arranged with maximum hydrophilic surfaces so as to have minimum free energy. Changes in protein solubility give direct evidence of the conformational changes of protein. Studies in *L.lenjan* and *R. kanagurta* showed increased protein extraction with increase in ionic strength up to 0.8 (Devi, 2006). Extreme pH conditions (pH 2 and 12) were also found to influence muscle protein solubility at least 5 times in comparison with extracts at pH 6-7 in *M. cephalus* (Mohan *et al.*, 2007). At pH above or below the isoelectric point, the protein acquires net negative or positive charges making them more hydrophilic exhibiting protein-solvent interaction than protein-protein interaction thus increasing protein solubility (Hamm, 1960). Howgate and Ahmed (1972) suggested that insolubility of muscle protein during heat treatment is related to the formation of inter and intra molecular links. During protein denaturation, the secondary and tertiary structure of proteins get altered due to breakage of both covalent and non-covalent interactions stabilizing the protein structure (Ngarize et al., 2004).

The biochemical and biophysical changes taking place during the development of *rigor mortis* influence significantly the functional properties of muscle proteins. Significantly higher protein content, enhanced gel forming ability and extractability are associated with pre-rigor tilapia (Park *et al.*, 1990). The post mortem drop in pH of the fish and the ensuing strong association of myosin and actins facilitate the solubility, which is markedly affected by temperature and duration of storage of the fish (Busch *et al.*, 1967; Chaudrary *et al.*, 1969; Shenouda, 1980; Reddy & Srikar, 1993). With increase in temperature the solubility decreases showing an inverse relation (Seki *et al.*, 1979; Kamal *et al.*, 1990). Alterations in solubility and functional properties have been observed during storage of fish in ice (Sarma *et al*, 1999; Parthiaban *et al*, 2005) and during frozen storage (Sarma *et al.*, 2000; Reynolds *et al.*, 2002). As the length of storage increases the solubility characteristics of the protein are altered and the content of both water soluble and salt soluble proteins has been decreased (Devadasan & Nair, 1971; Crupkin *et al.*, 1979; Reddy & Srikar, 1993; Parthiban *et al*, 2005). Pre processing conditions (Reddy & Srikar, 1991) freezing rate and storage temperature (Doong, 1988; Jiang *et al.*, 1988) influence the solubility of myofibrillar proteins. Moderate increase in protein solubility during the initial periods of storage in ice is attributed to the weakening of fibrous protein linkages in the muscle structure (Zayas, 1997). Insolubilisation of myofibrillar proteins is the major factor affecting the functional properties of fish protein during frozen storage (Borderias *et al.*, 1985). Aggregation of water soluble proteins, interaction between proteins and lipid oxidation/hydrolytic products, and protein – formaldehyde interaction are some of the factors contributing to protein insolubilisation during frozen storage (Ragnarsson & Reginstein, 1989; Ang & Hultin, 1989). The loss of extractability of protein during frozen storage is mainly related to changes in the properties of myosin, actin and actomyosin system (Dyer *et al.*, 1956; Matsumoto, 1980; Owasu-ansah and Hultin, 1992; Lin and Park, 1998). The changes in functional properties during frozen storage are attributed to increase in hydrophobicity due to unfolding of proteins as a result of storage at sub-zero temperatures (Shenouda, 1980).

Viscosity

Viscosity of proteins provides information on physico-chemical interaction among proteins indicating structural changes that may occur in the protein molecules (Kinsella, 1976; Pradipasena & Rha, 1977; Rha and Pradipasena, 1986). The measurement of viscosity provides useful information on shape and state of protein molecules (Prakash, 1982 and Shamasundar & Prakash, 1994). Viscosity by itself is not a functional property but an important intrinsic property of protein contributing to functional property. Viscosity is influenced by solubility and swelling properties of protein (Hermanson & Akesson, 1975). Highly soluble, non-swelling proteins possess low viscosity (albumin and globulins) while soluble proteins with high initial swelling show a concentration dependant decrease in viscosity (Kinsella, 1976). Change in apparent viscosity of muscle homogenates is related to changes in actomyosin (Borderias et al., 1985). This inherent property of protein decreases with increasing shear to which a protein is exposed. As shear is applied interactions between protein molecules gets weakened leading to breaking of the network thus decreasing the viscosity. Consequently any factors viz. pH, temperature, concentration and ionic strength, which unfold protein, influence their viscosity (Mohan et al 2007, Devi 2006). Viscosity is therefore considered as a more reliable index than solubility for protein denaturation (Colmenero et al., 1988).

Carp myosin is more sensitive to temperature, ionic strength and pH in comparison with chicken myosin (Nakayama *et al.*, 1975; Okada *et al.*, 1986). The viscosity of protein solution is directly related to protein concentration (Sarma *et al.*, 2000). The viscosity of protein solution markedly decreased during frozen storage due to protein denaturation and aggregation of protein molecules (Oguni *et al.*, 1987; Colmenero & Borderias, 1983; Colmenero *et al.*, 1988; Shamasundar & Prakash, 1994). Conformational changes in myosin molecule due to the temperature of storage expose the hydrophobic groups to the exterior resulting in sharp decline in the physicochemical properties of proteins (Roura *et al.*, 1995). The increase in apparent viscosity is an indication of protein-protein interaction and aggregation during thermal denaturation of proteins (Takashi *et al.*, 1993). There is significant correlation between viscosity and protein solubility in different fish during frozen storage (Colmenero & Borderio, 1983; Colmenero *et al.*, 1988).

Emulsion

Emulsions are thermodynamically unstable mixtures of immiscible liquids (Das & Kinsella, 1990; Zayas, 1997). Proteins capable of unfolding and exposing hydrophobic groups at the interface may exhibit this property and the ability of protein to emulsify fat is of great commercial importance (Xiong, 1997). Protein forms a thin film or coating on lipid droplet and provides an energy barrier to particle association and phase separation. When hydrophobic portions of a biological molecule particularly proteins is exposed to aqueous phase, they arrange themselves in to a form so as to have minimum exposure to water. Therefore, emulsion systems are stabilized by the interfacial protein film surrounding the fat globules leading to physical entrapment of fat within the protein matrix (Barbut, 1995).

In emulsion based food products the most important ingredient is often the emulsifier (Dickinson & Stainby, 1982; Damodaran, 1994) and the type of emulsifier largely determines overall appearance, texture and self-life of food emulsions. Myosin and actomyosin molecules by virtue of their structure and conformation due to both hydrophobic and hydrophilic residues act as excellent emulsifier (Galluzzo & Regenstein, 1978). The emulsifying properties of proteins are primarily due to their ability to reduce the interfacial energy at oil-water interphase. The emulsion capacity of a protein denotes

the maximum amount of oil that can be emulsified under specified condition (Zayas, 1997). Concentration and solubility of protein, pH of the medium, source and salt concentration affect the emulsifying capacity of proteins (Wang and Kinsella, 1976). There are reports indicating the differences in emulsion activity with size of the fish (Ramachandran et al., 2007) which may have direct relation to maturity as well. Factor such as equipment design, shape of container, rate of oil addition, and type of oil and nature of protein also influences emulsion capacity (Sasffle, 1968; Kinsella, 1976). Emulsion stabilization depends on the physicochemical properties of proteins and environmental conditions. Of the various intrinsic characteristics, surface hydrophobicity and the protein conformation have been suggested as important properties affecting protein stabilized emulsions (Kato & Nakai, 1980).

The emulsifying behavior of fish myofibrillar protein is better than that from chicken (Borderias *et al.*, 1985) and is related to the low stroma content of fish proteins (Matsumoto, 1980). There is a highly significant correlation between protein solubility and emulsion capacity in fish (Borderias *et al.*, 1985; Grabowska & Sikorski, 1974) and surface hydrophobicity of the myosin is the chief factor responsible for emulsion capacity (Chan *et al.*, 1984). Storage of fish in ice unfolds proteins exposing hydrophobic residues leading to increase in emulsion capacity compared to the original; however, emulsion capacity decreases in relation to protein insolubility during further storage (Parthiban *et al.*, 2005). The emulsion capacity of fish proteins is reported to decline during frozen storage (Careche & Tejada, 1990; Verma *et al.*, 1995; Sarma *et al.*, 2000) especially in species forming formaldehyde (Borderias *et al.*, 1985; Carech & Tejada, 1990; Huidobro & Tejada, 1993) and is attributed to protein-protein interaction or the interaction of protein with other degradation products formed during frozen storage.

The saroplasmic proteins from fish exhibited a higher emulsion activity at lower concentrations compared to myofibrillar proteins (Mohan *et al.*, 2006). The emulsion capacity of myofibrillar protein decreased with increasing protein content but emulsion stability shows a reverse trend (Mohan *et al.*, 2006). This decrease in emulsion activity with increasing protein concentration is reported to be due to greater degree of unfolding of protein during the shearing involved or due to the over saturation of the system with protein (Zayas, 1997).

Foaming

The formation of foam is analogous to the formation of an emulsion. Air is essentially non-polar and hence an ordering of the water molecules adjacent to the air cells occurs. This results in high surface tension and high surface energy. A protein that is utilized to form stable foam will require the same properties as are required to form an emulsion. The protein must be able to rapidly diffuse at the interface and unfold in such a manner as to lower the interfacial tension between air and water phase.

Foaming is a two-phase system in which air is suspended by a thin continuous liquid layer (Kinsella, 1981). During foaming soluble proteins diffuse to the air water interface, reduce the surface tension, unfold at the interface with orientation of polar moieties towards the water and interact to form the film with possible partial denaturation and coagulation. Protein solubility is the key requisite for foam formation and myofibrillar proteins from certain species exhibit good foaming (Baldwin & Sinthavais, 1974; Montero and Borderias, 1990) and chemically modified fish proteins showed considerably higher foaming capacity (Miller & Groninger, 1976). The foaming property of tilapia myofibrillar protein increased up to 10 days of iced storage of the fish and it is correlated with marginal increase in the hydrophobicity (Parthiban *et al.*, 2005)

Lipids inhibit foaming, even at low concentration (0.1 %), rupturing the protein film through increased surface tension, penetrating the inter-phase and displacing the proteins (Kinsella, 1981). The inhibitory effect of the lipids on foaming capacity diminish during frozen storage up to a certain level, perhaps as a result of changes in lipid protein interaction during storage (Careche. & Tejada, 1990). Foaming capacity is clearly affected by the formaldehyde generated during frozen storage (Huidobro & Tejada, 1992).

Water Holding Capacity

The water holding capacity of food refers to its ability to hold its own and added water during the application of forces such as pressing, centrifugation and heating. Hermansson *et al.* (1986) defined, Water Holding Capacity as a physical property and as the ability of a food structure to prevent water from being released from the three dimensional structure of the protein. Myofibrils are the largest water-holding filament lattices and most of the water in the meat is held within the myofibrils in the narrow channels between the filaments. Binding of water to the proteins is related to polar hydrophilic groups (Kinsella, 1982). Changes in protein conformation affect the thermodynamic property of water binding by affecting the availability of polar or hydration sites. The level of protein hydration and viscosity of liquid systems in food are interrelated. Consequently, protein water interactions determine functional properties of proteins in foods (Zayas, 1997).

The capacity of fish muscle to retain water is influenced by the species, sex and age, postmortem conditions, aging and processing conditions (Regenstein, 1984; Regenstein *et al.*, 1984). The storage of fish in ice leads to changes in water-holding capacity (Reddy & Srikar, 1993). Decrease in water retention capacity after two hours postmortem was observed by Kijowski *et al.*, (1982) and has been attributed to denaturation of proteins. Miller *et al.*, (1980) demonstrated a significant decline in the capacity of muscle proteins to retain water during extended frozen storage. Frozen storage decreased water holding capacity of fish protein due to denaturation and is supported by the increased thaw drip in frozen stored fish (Benjakul *et al.*, 2004; Wang & Xiong, 1998; Chang and Regenstein, 1997; Sarma *et al.*, 2000)

Gelation

Gelation is the association or cross-linking of protein polymer chains to form 3-D continuous networks, which immobilizes the liquid in the interstitial structure (Glicksman, 1982). Myosin or actomyosin participates in the gelling process of the meat through the formation of salt bridges, hydrogen bonds, disulfide bonds and hydrophobic interactions (Niwa, 1992). Salt solubilisation of myofibrillar proteins followed by partial denaturation by heating to form a regular network capable of immobilizing the water are the basic requirements to form good, firm and elastic gels (Suzuki, 1981; Lee, 1984; Lanier, 1986). Grinding of meat with NaCl, shift pH from isoelectric point of myosin and causes the exposure of hydrophobic groups to the protein surface resulting in loss of helical structure, creating more surfaces for protein –protein interaction. The dominant hydrophobic interaction among non-polar amino acid residues caused the myosin molecules to refold and re-aggregate. Subsequently, myosin regains its helical structure with the loss of solubility (Lin & Park, 1998).

The role of disulphide bonds in gel formation has been demonstrated especially in the case of actomyosin (Itoh, et al., 1979). Over a temperature range of 35 – 50°C greater hydrophobic surface area were exposed in cod and sliver hake myosin than in herring (Chan et al., 1992). In the setting process the a- helical structure of myosin and actomyosin unfolds markedly in the temperature range of 30 - 40°C (Ogawa et al.,

1995) and the exposed sulfhydryl groups are oxidized to disulphide bond during setting process. The unfolding of myosin at higher temperature is more intense and cysteine residues are more exposed which favour disulphide bond formation (Itoh et al., 1979). Gill and Conway, (1989) showed that thermal aggregation of myosin heavy chain was a result of non-covalent interaction, which get weakened in the presence of hydrophobic non-polar substances. The relationship between exposure of aromatic amino acid residues and extent of myosin aggregation provide indirect evidence for the role played by hydrophobic interaction in the self-association of myosin molecule (Chan et al., 1992).

Low temperature setting of fish mince influences molding and fiber development in surimi products (Niwa, 1992). Myosin heavy chain cross-links mediated by transglutaminase enzyme through g-carboxamide group of glutamic acid and ε-amino group of lysine (Seki *et al.*, 1990; Kimura *et al.*, 1991) favours setting of protein gels and this property has been commercially exploited for obtaining high gel strength products (Kimura *et al.*, 1991). High temperature setting of fish mince at 80 – 90°C results in strong gel with higher water holding capacity in comparison to a gel formed without cooking (Okada, 1959; Lanier, 1986, Kamath *et al.*, 1992). Hydrophobic interactions and non-sulphide covalent linkages play role in the development of gel network. (Niwa *et al.*, 1981; Wu *et al.*, 1985 a, b; Nishimoto *et al.*, 1987; Kim *et al.*, 1987 and Kamath *et al.*, 1992).

Loss of gelation has been attributed to denaturation, aggregation and autolysis of myofibrillar proteins during storage of fish in ice (Haard & Warren, 1985; Parthiban *et al*, 2005). Freshness of raw material is known to be one of the most important factors determining the gel-forming ability of the surimi (Lee, 1986). The rate of loss in gel strength appears to vary among species. Decrease in gel strength of kamaboko made from ice-stored lizardfish (Kurokawa, 1979) sardine (Leinot & Cheftel, 1990) threadfin bream (Yongswawatdigul & Park, 2002; Yean, 1993) hoki (MacDonald *et al.*, 1990) freshwater northern squawfish (Lin and Morrissey, 1995) and freshwater major carps (Sankar, 2000) have been reported and is related to changes in protein quality during storage.

Fish proteins have good physicochemical properties which vary depending on various parameters and play a crucial role in product development. Being labile, the structure of protein gets altered in relation to condition of exposure which in turn affect the functional properties either positively or negatively. Freshness of fish, type of processing, type and nature of storage etc. have a prominent role to play to determine the physicochemical property. The excellent physicochemical properties of the fish proteins are attributed to its unique three dimensional structures which facilitate the functional properties like solubility, water holding capacity, emulsion characteristics etc. Altering the three dimensional configuration by modifying the process and treatment can lead to changes in the inherent properties of fish proteins which help to develop high quality and high value products for human consumption.

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