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Gel Formation of Fish Structural Proteins Under Mild Acidic Conditions: Comparison With Conventional Surimi Gelation and Applications

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Washed fish meat homogenate in water could be converted into a gel by lowering its pH using weak organic acids such as acetic or lactic acid. Gelation results in imbibing of significant amount of water in the gel matrix of the proteins. The gel could be suitably diluted to get dispersions in which the fish structural proteins are highly soluble. Mild heating of the dispersion results in reduction of viscosity and its conversion into a free-flowing solution. Solubility of the proteins in the dispersion is not affected by temperatures as high as 100°C and/or high-speed centrifugation. However, the proteins are precipitated if traces of salt are added to the heated dispersions. The acid-induced gelation could be used to develop surimi-type restructured products from shark meat. Gel dispersions from shark and other fish species could be spray dried into protein powders. The dispersions can also be used as edible coatings to prevent quality loss in fishery products during chilled or frozen storage.

Key words: Weak acid induced gelation, surimi, protein dispersion, edible coating, protein powder

In recent times, importance of total utilization of fish landings, including under-utilized fish species as human food, has been realised because of the diminishing marine catch and increasing consumer interest in fish products. While several methods are available for value addition of fish, surimi technology has been recognized as one of the most successful techniques for low cost fish utilization. Surimi is myofibrillar protein concentrate produced by repeated washing of fish mince in order to remove water soluble nitrogenous and flavour compounds, and to enhance the gel forming capacity of the structural proteins. Surimi is used as a raw material for preparation of seafood analogues that possess the accepted texture, flavour and appearance of the natural products. This review discusses potential of gelation of fish meat in presence of weak organic acids, and points out the potential of such gelation and its applications in fish products development.

Conventional surimi gelation

A gel is an intermediate form between solid and liquid, in which strands of protein chains are cross-linked to form a continuous, three-dimensional network. Gelation involves essentially three steps, dissociation of myofibril structure by protein solubilization in the presence of salt, partial unfolding of myosin structure caused by heating and irreversible aggregation of unfolded myosin to form a three-dimensional structure (Lanier & Lee, 1992; Niwa, 1992; Sharp & Offer, 1992; Stone & Stanley, 1992). During aggregation,

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head portions of myosin molecules interact through disulphide bonds, and helix-coil transitions take place in the tail parts of the molecules (Stone & Stanley, 1992; Sharp & Offer, 1992). Several types of bonds are involved in stabilization of the gel structure, which include both covalent (disulphide) and non-covalent (hydrophobic, hydrogen bonding and electrostatic) linkages. During the aggregation process, water, oil and flavouring compounds may be entrapped in the gel matrix. Thus, rigidity of the gel could be manipulated by incorporating suitable ingredients.

Surimi has a neutral or slightly alkaline pH. In the classic surimi gelation, partial denaturation of the fish proteins is achieved by mild heating. An amount of 1.5 to 3% salt is added to the surimi, which helps dissolution of the proteins. Gelation is observed when surimi is incubated at 0° to 4°C for 12 to 18 h, 25°C for 2 h or 40°C for 30 min, for low, medium or high temperature settings, respectively. The low temperature set gel is weak and has high elasticity and transparency. After the initial heat treatment, the surimi is heated at 90°C for 15 min, which makes the gel opaque and rigid.

Gelation of fish structural proteins by weak acids

Acid-induced gelation also requires thorough washing of the fish meat, as in the case of conventional surimi, which is then subjected to controlled lowering of pH to induce gelation. Organic acids, especially, acetic and lactic acids are the common acidulants used. These are weak acids having ionisation potential ideal to slowly lower the pH. Inorganic acids cannot be used since they cause drastic lowering of pH causing protein precipitation. The proteins, which unfold as a result of mild acidification, aggregate among themselves to form gel. The aggregation and gelation could be enhanced when the pH-lowered fish proteins are subjected to heat treatment (Venugopal *et al*, 1994a). Prior to acidification and heating, as in conventional surimi, various additives could be incorporated in order to modify the texture of the aggregated proteins. However, it should be remembered ionic compounds including common salt interfere with the gelation process at acidic pH. Therefore, for the product formulation, these additives need to be added only after the gelation process is completed. Table 1 gives a comparison of conventional surimi gelation with weak acid induced gelation.

Preparation of weak acid induced fish meat gel

For preparation of gel, the fish mince or meat pieces are subjected to repeated thorough washing in cold water, as in the case of conventional surimi preparation . In the case of fatty fish such as mackerel and herring, it is advisable to use a 0.5% aqueous solution of bicarbonate in the second washing in order to remove adhering lipids. The washed meat obtained is homogenized, generally in equal amount of cold water, using a kitchen homogenizer. To induce gelation of the washed meat, glacial acetic acid is added drop wise to the slurry while stirring it gently, to reduce the pH to about 3.5. Usually acetic acid at concentration of 0.5% of the slurry is sufficient for reducing the pH to 3.5. In the case of homogenate of washed shark meat in water, the pH induced gelation of the protein is indicated by thickening of the slurry, which could be observed visually. The rate of increase in viscosity is slow, if the slurry is cold. It can be accelerated by gentle warming. The final viscosity of the product is dependent upon meat (protein) content of the slurry. The moisture content of the gel varies between 88 to 93% when washed shark meat is homogenized with water in a meat to water ratio of 1:1, 2:3 or 1:2, respectively, with

Table 1. Comparison of conventional surimi gelation with weak-acid induced gelation of fish structural proteins.

Characteristics	Conventional surimi gelation	Weak acid induced gelation
Gelation pH	Neutral or slightly alkaline	Acidic pH in the range of 3.5 to 4.0
Agents for gelation	Mild heat in presence of NaCl	Weak organic acids such as acetic or lactic acid
Retention of water	Water retained in the gel	Water retained in the gel
Chemical changes	Formation of covalent (disulfide) and non-covalent linkages, degradation of myosin heavy chain. Decrease in α helix content and increase in hydrophobicity of myosin.	Formation of covalent (disulfide) and non-covalent linkages, degradation of myosin heavy chain. Changes in α helix content and hydrophobicity of myosin?
Microbial stability	Poor microbial stability	Good microbial stability
Rheological characteristics	Visco-elastic nature of surimi gel from several fish species	Retention visco-elastic nature in the case of only two fish species (shark and Alaska Pollock) reported
Influence of ionic compounds on the gel	Visco-elasticity not affected	Adversely affect gelation and retention of water
Influence of heat on gel	No significant changes in viscosity	Rapid fall in viscosity of gel giving free-flowing dispersion. Solubility of proteins in dispersion not affected
Applications	Restructured products	Restructured products, edible coatings for fish, spray dried powders and other potential applications

respective gel strengths of 134, 147 and 158. However, as compared with conventional surimi, the values are lower suggesting weaker nature of the gel (Venugopal *et al*, 2002b). Instead of acetic acid, lactic acid could be employed, while citric, tartaric or hydrochloric acids were ineffective. Furthermore, presence of NaCl was detrimental to mild-acid induced gelation of shark meat proteins, as will be discussed later (Venugopal *et al* 1994a). In the case of Bombay duck, because of the high lability of the proteins, the washed meat is held overnight in a cold room in presence of 0.5% acetic acid to induce gelation (Kakatkar *et al*, 2003).

Unlike shark meat, gelation by acetic acid of washed meat of threadfin bream, Bombay duck, Indian mackerel, Atlantic mackerel and freshwater fish, rohu, was not characterised by thickening or increase in viscosity of the meat slurry (Venugopal, 1997). Instead, there was a fall in viscosity of the slurry. Table 2 shows the influence of mild acidification by acetic acid on the apparent viscosities of washed fish homogenates in water. This difference in behaviour between shark and other fish species could be as a result of difference in the type of myosins in the fish. In the case of capelin, gelation was observed after thorough washing of the meat followed by mild heat treatment of the washed meat slurry in water. Acetic acid addition was not required for gelation (Venugopal *et al*, 1995). In the case of all the fish studied, the acid-

Table 2. Influence of acetic acid on the apparent viscosity of washed fish homogenates in water

Fish species	Protein content of water homogenate (%)	Viscosity, without acetic acid	Viscosity after addition of acetic acid (pH, 3.5)	Viscosity after acetic acid addition followed by heating at 50°C.
Atlantic mackerel	2.4	5.9	0.3	0.06
Atlantic herring	1.6	3.4	1.4	0.07
Indian mackerel	1.5	4.0	0.5	0.05
Capelin	1.9	3.0	A	0.04 (no acetic acid required)
Threadfin bream	2.3	6.2	1.2	0.04
Shark	2.3	12.0	600	>1200

The viscosity values are denoted as apparent viscosity values, and expressed as Pa.s. Viscosity of shark was measured using Brabender viscoamylograph and expressed as Brabender units. A, samples precipitated in presence of acetic acid.

treated washed meat could be suitably diluted with water to get dispersions of the meat.

Entrapment of water in the gel matrix

Washing and acid induced gelation are associated with hydration of the fish proteins during washing and also acid induced gelation. In the case of all the fish species examined including shark meat, and rohu, water was firmly entrapped in the gel matrix (Panchavarnam, *et al*, 2003). The bound water could not be separated from the meat by heat treatment and/or high speed centrifugation. Thus, while centrifugation of unacidified shark slurry resulted in separation of as much as 50% water, no water was separated from the hard mass even when centrifuged at 12,100 x g for 30 min. This was also true when the gels were subjected to heat treatment at temperatures as high as 100°C. Heating nevertheless, converted the gel into a free-flowing dispersion. However, addition of traces of ionic compounds including common salt to the gel prior to heating resulted in total collapse of the protein network and separation of water, as will be discussed later. Usually, an expressible water content not exceeding 10% is found in the case of surimi (Lanier & Lee, 1992). However, in the case of mild acid induced shark gel, expressible water was above 30%, suggesting comparatively weak nature of shark gel. The strong binding of water in the gel matrix, nevertheless, suggest that mild acid induced gelation could be used to prepare thermostable dispersions of meat from various fish species. These dispersions can have potential applications, as will be discussed later.

Chemical changes during gelation

Mild acid induced gelation of fish meat is associated with decrease in sulphydryl contents and formation of disulphide bonds. This has been verified with respect to shark and threadfin bream gel (Chawla *et al*, 1996). Decrease in SH groups in the proteins is comparable with conventional surimi gelation (Stone & Stanley, 1992). Another change is the disappearance of myosin heavy chain (MHC). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) data indicated that while the unacidified meat was characterized by predominant band of MHC, it was feeble in the case of the gel, suggesting degradation of MHC during gel formation (Venugopal *et al.*, 2002b). Acid-

induced gelation of threadfin bream resulted in MHC and appearance of a protein band of about 186 kDa. Solubility of the gel in various solvents containing sodium dodecyl sulphate, urea and b-mercapto-ethanol suggested structural changes in the proteins during gelation (Chawla *et al.*, 1996). Changes in hydrophobicity and a helix conformation of myosin molecules, which are known to be occurring during surimi gelation, have not been well studied with respect to acid induced gelation.

Viscoelastic nature of shark gel

As compared with conventional surimi, acid-induced shark gel is weak. Nevertheless, the acid gel is also characterised by viscoelastic property, as revealed by dynamic rheological measurements (Venugopal *et al.*, 2002b). The shark gel showed by a higher storage modulus, G′ than loss modulus, G″. The G′ increased with decrease in moisture contents suggesting higher rigidity of the gels. The G′ and G″ profiles indicated structural changes in the gels at temperatures above 50°C. The elation process could also be monitored by measuring changes in stress-strain phase angle during oscillatory testing. The relatively low tan (G″/G′) values indicated elastic nature of the gel (Venugopal *et al.*, 2002b). Strength of the gel, for example that of threadfin bream, was influenced by setting conditions and heat treatment (Chawla *et al.*, 1996).

Water dispersions of acid induced-fish meat gel

The muscle structural proteins including myosin and actomyosin are sparingly soluble in water, requiring extractants having a high ionic strength for their dissolution. Furthermore, if solubilized, the solutions are highly viscous in nature. In addition, the proteins undergo rapid denaturation even under mild heating conditions. These properties limit applications of fish meat for product development. Ideally, such water dispersions of fish muscle structural proteins should be of low viscosity (free-flowing) and also stable against heat-induced denaturation and precipitation. The ability of conversion of fish meat into acid-induced gels favours development of thermostable, free-flowing, water dispersions of meat from several fish species. The gel could be suitably diluted with water in which the proteins are highly soluble. Fig.1 shows general process for preparation of weak acid-induced gel and protein dispersion from fish meat.

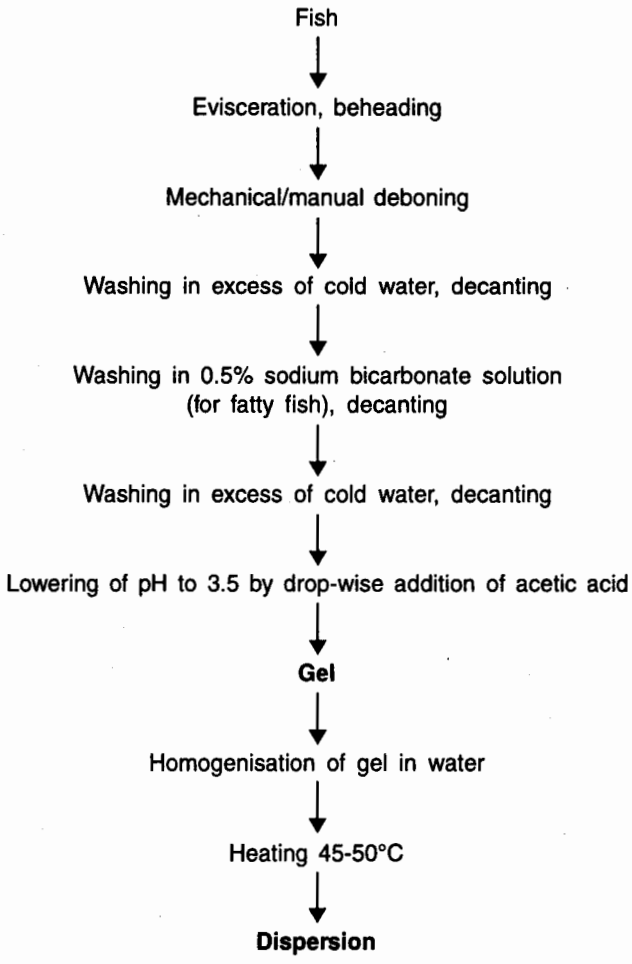

Fig. 1. General process for preparation of weak acid induced gel and thermostable water dispersion of fish meat

The proteins in the dispersions are not precipitated by a combination of heating (100°C, 15 min) and centrifugation (up to

135,000 and X g), as shown in Table 3. However, almost all of the proteins in the dispersions are precipitated when the preparations were heated after increasing the pH from 3.5 to 7.0. The presence of salts such as NaCl and $CaCl_2$ adversely affected stability of proteins in the dispersions (Venugopal, 1997). This suggested that the water is entrapped in the network as a result of mild acid induced electrostatic interactions in the protein network. Disturbance of the electrostatic interactions by the ionic compounds interfered with the stability and collapse of the gel. The mechanistic aspects of stability of the proteins in the dispersions have been discussed elsewhere (Venugopal, 1997).

Table 3. Protein content of water homogenates of washed fish meat and thermostable gel dispersions

Fish species	Protein content of homogenate (%)	Protein content of gel dispersion (%)	Solubility of proteins in dispersions (%)
Atlantic mackerel	2.4	2.35	98.0
Atlantic herring	1.3	1.28	99.0
Indian mackerel	1.5	1.3	86.0
Capelin	2.5	2.1	84.0
Shark	2.94	2.16	73.0

Rheological (viscosity and flow) properties of the dispersion have been studied in the case of fish species including Bombay duck, capelin, Atlantic mackerel, Atlantic herring, capelin, Indian mackerel, and threadfin bream. Washed capelin mince homogenate in cold water showed higher apparent viscosity depending upon protein concentrations. Influence of shear rate on the apparent viscosity suggested psueoplastic nature of the dispersion (Venugopal *et al.*, 1995). The apparent viscosity was significantly reduced by heating to 35° to 40°C. However, solubility of the proteins was not affected. Most of the proteins were retained in the solution (Table 3) (Venugopal *et al*, 1994b; 1995; 1997). A low viscous, thermostable gel could be prepared from shark gel at 3% protein concentration, which was suitable for spray drying (Venugopal *et al*, 1997). Apparent viscosity of washed Bombay duck muscle in water showed initial increase at medium temperatures followed by rapid fall at higher temperatures (Kakatkar *et al*, 2003). Rheological studies on other fish protein dispersions from acid-induced gels include those on Atlantic herring and Atlantic mackerel (Venugopal & Shahidi, 1994; Shahidi & Venugopal, 1994).

Application of weak acid induced gelation of fish proteins

Surimi type restructured products from gel

Our recent studies indicated that the phenomenon could be applied for value addition of shark meat through development of restructured steaks (Venugopal *et al.*, 2002a). The shark gel obtained, as mentioned above, is filled in desired moulds, steamed for 15 min, aerobically packaged in polyethylene pouches and stored at refrigerated temperatures. Because of the presence of acetic acid, the product is also stable against microbial growth when stored for 2 months at 10°C. Prior to consumption, the product is deacidified and salted to taste by dipping in an equal volume of aqueous solution of sodium bicarbonate (baking soda) and common salt at concentrations of 5% each for 20 min. Consumer studies indicated good acceptability of the product. Alternately, the shark gel, cut into desired shapes, could be used as paneer (Venugopal *et al.*, 2002a).

Acid induced gelation has been recently extended for development of low sodium surimi from Alaska Pollock (Lian *et al*, 2002).

Physical characteristics such as shrinkage, total expressible fluid and firmness, of the acid-induced Alaska Pollock surimi were influenced by concentration of acetic acid, used for gelation. In addition, acid induced gels without starch were significantly firmer than those with starch and salts.

Preparation of functional protein powders from gel dispersions

Fish structural proteins in solution are highly unstable to heat and sensitive to denaturation and precipitation. Therefore, attempts to prepare functionally active fish protein powder by spray drying have not been successful. Niki *et al.*, (1982) partially solved the problem by dissolution of Alaska Pollock meat surimi through milling in presence of sorbitol and carbonic acid. The acid was incorporated to reduce the high viscosity of the solution. The powder obtained contained 65% protein and high content (24%) of carbohydrate. In the case of gelation-dependent thermostable water dispersions, the solubility and stability of proteins in the dispersions at high temperatures could be taken advantage of for the development of functionally active protein powders by spray drying. Since there was no need for any additive such as carbohydrate, as mentioned above, the powder prepared had more than 90% proteins (Venugopal *et al*, 1994b; 1996). As mentioned above, proteins in the dispersion precipitate in presence of traces of salt. This phenomenon could be employed for concentration of the proteins by techniques other than spray drying.

Use of dispersions as edible coating to extend chilled shelf life of fresh fish

Currently there is increasing interest in biodegradable films and coatings for food applications. Several potential and innovative applications of edible films to improve overall food quality, such as extending shelf life and possibility of cost reduction of packaging materials have been envisaged (Krochta *et al*, 1994; Gennadios *et al*, 1997). Edible coatings from biopolymers including proteins have been developed (Gennadios *et al*, 1997). The properties of these films including tensile strength, moisture permeability etc. vary widely (Krochta *et al*, 1994). It has been recently proposed that thermostable dispersion of shark myofibrillar proteins could be suitably converted into edible coatings, which could be used as a coating for commercially important fish items (Venugopal, 1998). Cuq *et al.* (1995) reported preparation of edible packaging films based on gelation of fish myofibrillar proteins at highly alkaline pH. Edible coating was prepared in our laboratory from acid-induced gel dispersion from rohu (*Labeo rohita*), which could be used to enhance the chilled storage life of the fish. Gel from rohu was prepared by the method described above. The dispersion was employed as coating for fresh steaks of rohu itself. Dipping of the steaks for 1 h in the dispersion, followed by packaging in polyethylene pouches and chilled storage gave a shelf life of 32 days as compared to about 20 days for the non-coated steaks under the same conditions (Panchavarnam *et al*, 2003). The extension in chilled storage life could be due to acidic nature of the dispersion and presence of acetic acid, which is a known antimicrobial compound (Lin & Chuang, 2001). However, the acidic nature of the dispersion could cause some bleaching of the fish pigments during storage. This could be prevented by incorporation of 0.5% of either butyl hydroxyanisole or ascorbic acid in the dispersion as antioxidant (Panchavarnam *et al*, 2003). Similarly, coating of seer fish steaks by dispersion prepared from the same fish could enhance chilled storage life of the fish steaks (unpublished data).

Fish based edible coatings could also be used to prevent moisture loss, lipid oxidation

and discolouration during storage of frozen fishery products. Recently we have observed that edible coatings from mackerel could be used as coating of mackerel mince blocks to prevent weight loss due to dehydration and rancidity development during prolonged frozen storage. In addition, about 50% reduction in TBA value was noted in the case of protein glazed mackerel blocks, as compared with non-glazed blocks (unpublished results).

Fish sauces from gel dispersions

Fermented fish sauces are delicacies in several countries, particularly in Southeast Asia. Traditionally these are prepared by fermentation of the whole fish, making use of the enzymatic activity of fish viscera, activities of extraneously added microorganisms or both. (Venugopal & Shahidi, 1995). Highly hygienic as well as suitably flavoured fish sauces could be prepared by fermentation of weak acid-induced fish gel dispersions by lactic or propionic acid bacteria. Preliminary studies have indicated possibility of preparation of such sauces from shark meat gel (Sree Rekha *et al*, 2002). Another application is development of microbiologically stable pattice and sausages, that can have extended chilled storage life through incorporation of weak-acid induced gel (unpublished results). Potential also exists for use of the gel as binder in aquafeed to enhance its water stability.

Conclusions

Fish muscle structural proteins could be converted into a gel by controlled lowering of pH by weak organic acids such as acetic or lactic acid. The gel could be applied for development of restructured products incorporating suitable additives. There are potential for applications of the gel as binder in sausages and aqua feeds. The gel dispersion could be used as a coating in order to enhance chilled storage life of fresh fish and to prevent quality loss during frozen storage of fishery products.

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