7

Chemical processing methods for protein recovery from marine by-products and underutilized fish species

H. G. Kristinsson, A. E. Theodore and B. Ingadottir, University of Florida, USA

7.1 Introduction

Fish has an amino acid composition which makes it an excellent source of nutritive and easily digestible protein. Fish proteins also possess properties that make them good agents of water holding, gelation, fat binding, emulsification and foaming (Xiong, 1997; Kristinsson and Rasco, 2000). For these reasons they are an attractive food source and ingredient for various food applications. The great demand for quality fish protein in the world is growing at a faster pace than can be met with traditional resources, and has in many places led to significant over fishing, often requiring governmental intervention (Kristinsson and Rasco, 2002). Despite the current bleak situation and the economic disruption this has caused, there are still abundant sources of fish that are underutilized in the sense that they are not utilized as human food, most notably the fatty pelagic fish species and processing by-products. The underutilized species are normally small dark muscle pelagic fish species, and make up to 40–50% of the world fish catch (FAO, 2000). Only about 40% of the small pelagic species caught are utilized for human consumption (Unido, 1990). The potential exists to develop high-value functional protein products from these species. Furthermore, many fisheries operations lead to substantial amounts of by-catch consisting of a complex array of different fish species. For example, it has been reported that over four pounds of by-catch is caught by shrimp boats in the Gulf of Mexico for every pound of shrimp using conventional fishing gear (Cushman, 1998). This material is typically discarded back to the sea with little attempt at recovery, and represents an enormous amount of high quality protein which can be utilized for

human consumption and furthermore could create a substantial dividend for the local fishing industry. Even with the best preventative gear available to reduce by-catch it will inevitably always be a sizable portion of the catch or about 2–3 pounds fish per pound of shrimp (Gulf and South Atlantic Fisheries Foundation, Inc, personal communications). Discarding the product makes little economic and environmental sense. The same holds true for fish processing by-products which are typically composed of fish frames generated from filleting, including visceral material, and are usually discarded or utilized in animal feed or fertilizer.

Using conventional technologies to process fish and creating value added fish products generally leads to limited utilization of the animal and large amounts of protein-rich by-product materials are lost and not recovered. For example, even the most efficient filleting operations will always yield great amounts of protein-rich by-products, up to 60–70% of the fish depending on species (Mackie, 1982; Kristinsson and Rasco, 2000). This material is high in quality protein and lipids, and other valuable compounds which could be utilized for human consumption. The global aquaculture industry is also growing at a rapid rate and should not be overlooked as it will lead to more processing by-products in the coming years, which could provide a sizable source of quality food protein and lipids.

The advantages of a process aimed at isolating high-quality food protein from underutilized fish species and by-products are obvious. To upgrade products made from pelagic species and by-products would not only add economic value and assist the seafood industry, it would also be a more responsible use of these sources. Major efforts have been undertaken in both academia and industry in the past century to reach the goal of economic recovery and utilization of proteins from underutilized species of fish and byproducts (Kristinsson and Rasco, 2000, 2002). Most of these efforts have been met with limited success. Some of the key hurdles in the successful and economic recovery of fish proteins include: (a) the processes have to be able to process the material with as little pre-processing as possible (ideally whole fish), (b) the processes have to be able to utilize low-value sources of fish such as fatty pelagic fish species, trimmings and frames, and (c) the processes have to be able to yield a consistent, functional, palatable and stable product (Kristinsson and Rasco, 2000, 2002). Pelagic species, by-catch and by-products present the fish processor with numerous difficulties with respect to their utilization. These raw materials are very complex, including bones, skins, connective tissue, abundance of oxidatively unstable lipids, large amounts of pro-oxidants (blood and heme proteins), unstable muscle proteins of low functionality and in some cases high levels of active proteases (Okada, 1980; Hultin, 1994; Hultin and Kelleher, 2000). The above factors hamper their direct consumption and greatly limit the possibilities to economically recover functional proteins from them using conventional techniques. Various attempts have been taken towards this goal in the past but with limited success. Both chemical and enzymatic processes have been developed with the goal to recover functional ingredients from these materials, most notably proteins and lipids. One of the oldest methods to recover proteins

from fish muscle is surimi processing which includes washing ground fish muscle with water to leech out undesirable water soluble proteins and lipids. This approach, however, has limited utility on very complex raw materials such as whole fish or by-products. Early attempts on complex raw materials included harsh chemical extraction processes where both proteins and lipids could be effectively extracted, but in many cases functionality was lost and palatability was low. This led to a growing interest in the production of fish protein hydrolysates, which are extracted with enzymes under milder conditions, and are discussed in detail in Chapter 10. Using proteases to extract fish proteins does, however, lead to modifications in their functionality, and often a reduction in certain key functional properties. Therefore, there was still a need to develop a mild chemical extraction process, which could be used on challenging raw materials but not compromise protein functionality. This has recently been achieved with a novel acid and alkaline solubilization/precipitation process developed by Hultin and coworkers (Hultin and Kelleher, 1999; Hultin *et al.*, 2004). This chapter discusses some of the principal chemical processes developed primarily with the goal of extracting functional fish proteins.

7.2 Chemical extraction: fish protein concentrate

One of the earliest attempts to recover protein from by-products and under-utilized species for use as a human food was the production of fish protein concentrates (FPC). Fish protein concentrates are produced by using chemical solvents and sometimes high temperatures to extract and separate proteins from other components of the raw material (e.g., fat). The National Marine Fisheries Service (NMFS) in the US (then Bureau of Commercial Fisheries) initiated a large research program in this area in the early 1960s with the goal of finding ways to produce FPC on a large scale to stimulate the US seafood industry and also fight the global protein malnutrition problem (Snyder, 1967). The process of making FPC is relatively straightforward. Solvent extracted FPC (type-A FPC) is produced by extraction with isopropanol or azeotropic extraction with ethylene dichloride. Ethanol has been successfully used as well. Figure 7.1 shows one example of FPC processing (Sikorski and Naczk, 1981). The raw material is ground and then extracted with isopropanol at 20–30ºC for 50 minutes. The supernatant is then collected and extracted two times, first at 75ºC for 90 minutes with isopropanol and then at 75ºC for 70 minutes with azeotropic isopropanol. This gives a final supernatant fraction which is then dried, milled and screened to separate out bone pieces. The final product should be largely colorless and odorless and primarily consist of protein (<1% lipids) with high biological value. This relatively harsh process does, however, take its toll on the functionality of the proteins. Type-A FPC is poorly soluble or dispersible in foods, which greatly limits its applicability (Cheftel *et al.*, 1971; Mackie, 1974; Venugopal *et al.*, 1996). Relatively poor emulsification properties have also been reported (Cheftel *et al.*, 1971; Mackie, 1974; Venugopal *et al.*, 1996).

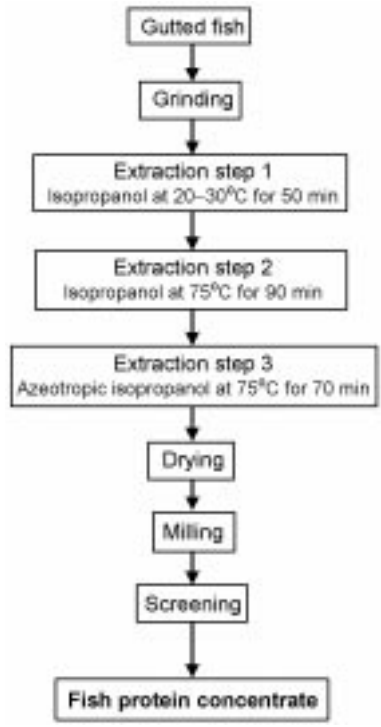

Fig. 7.1 An example of fish protein concentrate production using isopropanol as the extracting solvent (adapted from Sikorski and Naczk, 1981).

Temperature during extraction has an impact on the functionality of the protein. For example, it has been reported that FPC produced at 50ºC had significantly lower emulsifying properties compared to FPC produced at 20ºC (Dubrow *et al.*, 1973). Both had very low solubility. Some studies have, however, reported that FPC has good foaming properties over a wide pH range (pH 2–11), although this functional property may be of limited interest for a fish protein ingredient (Sheustone, 1953; Hermansson *et al.*, 1971; Kinsella, 1976). Despite major problems with protein functionality, solvent extraction has been the method of choice for fatty pelagic fish species (e.g., sardine, herring and capelin) since the protein is effectively separated from the oil, thereby improving oxidative stability. It has been reported that isopropanol is a slightly more efficient solvent than ethanol for fatty fish species since it removes more oil (Moorjani *et al.*, 1968). However absolute ethanol was able to produce FPC of lighter color and a more neutral flavor (Moorjani *et al.*, 1968).

Very few recent studies have been reported on the production and use of FPC, since more successful protein extraction techniques are now available (e.g., fish protein hydrolysates (Chapter 10) and fish protein isolates made with pH-shift processing, see below). There are, however, a handful of papers from the last 10–15 years which demonstrate that solvent extracted FPC may find good uses if

it is produced properly. For example Vareltzis and coworkers (1990) used ethanol extraction to make FPC from sardines and added it to hamburger patties. These authors reported that that the overall functional properties of the hamburger, i.e. water binding and cooking yield, increased with addition of FPC. Penetration depth and shear force value of the FPC added hamburger also indicated a better hamburger patty. However, on the downside, the hamburgers were found to have a slightly unfavorable fishy flavor.

Although FPC on its own may possess poor properties in many cases, several studies have shown that FPC may be a good substrate for enzymatic hydrolysis to make fish protein hydrolysates (FPH) (Cheftel *et al.*, 1971; Hale, 1972; Spinelli *et al.*, 1972; Quaglia and Orban, 1987a,b; Hoyle and Merritt, 1994). This is because it provides a largely oil-free substrate and has partially denatured proteins which are highly susceptible to enzymatic hydrolysis (Kristinsson and Rasco, 2002). Enzymatically hydrolyzed FPC have generally greatly improved solubility and dispersibility compared to the parent FPC, while some other functional properties such as foaming would be reduced (Hermansson *et al.*, 1971; Kristinsson and Rasco, 2002). Taste and odor problems are generally minimized for FPH when FPC is the starting material (Hale, 1972). For example, Hoyle and Merritt (1994) found that FPC made from herring with ethanol extraction and then hydrolyzed with either Alcalase or papain produced a FPH with a reduced bitterness and less fishy odor compared to FPH made directly from the herring. However, general poor functionality, off-flavors and colors, high cost of production and possible traces of solvent in the final product have made solvent extracted FPC commercially unsuccessful regardless of intensive efforts (Mackie, 1982).

7.3 Chemical hydrolysis

Proteins can be chemically hydrolyzed with either acid or base with the help of high temperatures. Chemical hydrolysis is a relatively inexpensive and simple method to extract fish proteins from by-products and several processes have been proposed for the acid or alkaline hydrolysis of fish (Hale, 1972). Chemical hydrolysis is, however, a difficult process to control and because of that leads to end products with variable composition and functionality (Blenford, 1994; Skanderby, 1994). Furthermore, hydrolyzing proteins at very low or high pH, sometimes in the presence of chemical solvents at very high extreme temperatures, generally yields products with reduced nutritional qualities, poor functionality and restricts their use to products such as seafood flavorings (Webster *et al.*, 1982; Loffler, 1986).

Acid hydrolysis is a more commonly used method to hydrolyze fish proteins than alkaline hydrolysis. Acid hydrolysis of fish protein normally involves adding strong hydrochloric acid or sulfuric acid to the fish raw material and then extensively hydrolyzing the proteins at a high temperature, sometimes under high pressure. Total hydrolysis can be achieved in 18 hours at 118ºC in 6N

hydrochloric acid (Thomas and Loffler, 1994), although those conditions would rarely be used. The resulting fraction containing the hydrolyzed proteins is then neutralized to pH 6.0–7.0 and dried or concentrated (Thakar *et al.*, 1991). The extensive hydrolysis leads to a product of very high solubility and dispersibility, while other functional properties are largely destroyed (Kristinsson and Rasco, 2002). Due to the pH neutralization, the hydrolysate can contain a large amount of salt (NaCl) which can reduce the palatability of the product. Another downside of the acid hydrolysis process is the destruction of tryptophan, which is an essential amino acid. This limits its use as a protein ingredient in food or animal feed.

A handful of publications have shown that acid hydrolysis may find a use as a protein recovery process. Orlova and coworkers (1979) reported a relatively promising process where they used acid hydrolysis on whole fish, followed by steam distillation to remove aromatic substances, then filtering and concentrating the extracted and hydrolyzed protein. The concentrate was successfully used in dehydrated soup cubes and as a microbial media (Orlova *et al.*, 1979). Acid hydrolysis (sometimes with the aid of acidic proteases) is also commonly used to convert underutilized species and processing by-products into fertilizer, due to the simple operation, low production cost and extensive hydrolysis which makes the peptides/amino acids easily utilized by plants (Kristinsson and Rasco, 2002).

The use of alkali (mainly sodium hydroxide) to hydrolyze protein can result in poor functionality and adversely affect the nutritive value of the final product. Despite these drawbacks, limited alkali treatment is used in the food industry to recover and solubilize a broad range of proteins (Kristinsson and Rasco, 2002). Very few studies have been published on the alkaline hydrolysis of fish proteins. One of the reported key benefits of alkaline hydrolysis is to help modify and improve functional properties of otherwise highly insoluble FPC (Sikorski and Naczk, 1981). For example, Tannenbaum and coworkers (1970a,b) developed a small-scale batch process that utilizes very high pH (12.5) and 95ºC for 20 min. The final product consisted of large peptides, some which were relatively insoluble at the isoelectric point of myofibrillar proteins, but demonstrated an overall improvement in functionality compared to the original FPC. These authors reported that the alkaline-treated FPC could be used as a milk substitute, giving a product of far superior properties to that obtained with the original FPC, which had very low solubility and dispersibility.

7.4 Surimi processing

Surimi originated in Japan where it has been a traditional food source for centuries. Currently, Japan consumes about 70% of the surimi produced worldwide. In Japan, the popular surimi-based products include: satsuma-age, chikuwa, kamaboko, flavored kamaboko, hanpen/naruto, and crab sticks. Imitation crab chunks, flakes, and sticks are the most popular form of surimi consumed in the

United States and Europe where it's gaining much popularity, and consumption is growing at a rapid rate every year and the product receives a premium price (Anonymous, 2001). China, Russia, and South America have also recently discovered surimi-based crab sticks and are becoming major users (Park, 2000).

Surimi once referred to the ground fish paste formed during the manufacturing of the surimi-based product kamaboko. Surimi now describes mechanically deboned then washed fish muscle which is used as an ingredient for a range of imitation seafood products, primarily crustacean and shellfish substitutes. It is important not to confuse fish mince with surimi. Fish mince is a starting material of surimi, not surimi itself. Briefly, in conventional surimi processing the raw material is minced, mechanically deboned, washed with water, strained, dewatered, cryoprotectants added and the product packaged and finally frozen in blocks until used. The remaining myofibrillar protein concentrate demonstrates enhanced functional properties, such as gel-forming ability, water holding capacity, and fat-binding (Okada, 1992). Surimi is often modified for long-term storage or further processed into other seafood products, such as imitation crab meat, by incorporating additional components such as flavoring agents, sugars, and salts. The primary fish species used to make surimi in Japan and in the United States is Alaskan pollock. However, other species such as menhaden (*Brevooritia tyrannus*), red hake (*Urophycis chuss*), Pacific whiting (*Merluccius productus*), and spiny dogfish (*Squalus acanthias*) are being used in the surimi industry (Gwenn, 1992). For many years the industry was dependent on supply and availability of fresh fish. However, the discovery of adding cryoprotectants to surimi in order to prevent protein denaturation during freezing revolutionized the industry (Park and Lanier, 2000) which was no longer dependent on fluctuations in supply of fresh fish.

Surimi manufacture is a multi-step process, as shown in Fig. 7.2. Fish heads are removed, guts are cleaned, and bones are removed with large amounts of water to separate the waste material from the muscle tissue. The muscle is then minced by passing the material through a perforated screen and collecting the mince. During the mincing process, tough cartilage, skin, and bones do not pass through the mesh screen, thus removing further undesirable material from the muscle. By removing blood, skin, membranes, and other materials, the muscle becomes more stable and yields a higher quality product (Park and Morrissey, 2000). The next phase in surimi processing is the washing step. The number of washing cycles and water volume depend on many factors, such as fish species, facility type and capacity, initial fish quality, and desired final surimi quality. Generally, the fish to water ratio is between 1:5 and 1:10, although more modern operations are able to use ratios as low as 1:2. Washing with water removes components that can have negative effects on gelation (e.g., sarcoplasmic proteins, although this is debatable) and compounds that can cause flavor, odor, stability and color problems.

The product after washing primarily consists of myofibrillar protein, with a significant decrease in amount of blood, soluble proteins, connective tissue and fat (which are mostly removed during washing) as compared with the starting

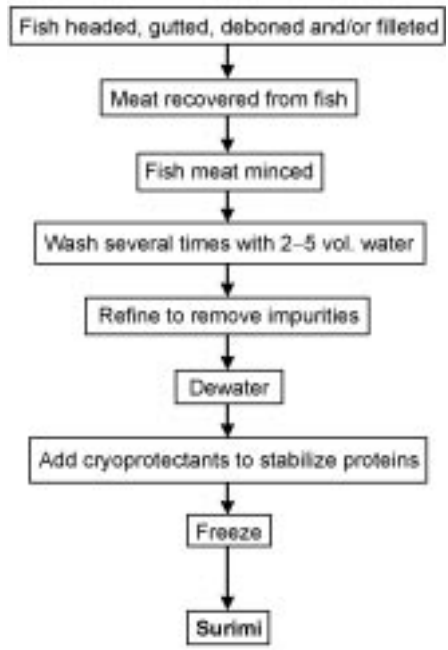

Fig. 7.2 Processing steps in conventional surimi processing.

material. By removing or at least decreasing the amount of undesirable compounds in the fish, the surimi texture, color, flavor, and storage quality is increased. During the entire process the temperature should be maintained low enough to prevent protein denaturation which varies according to species (Ohshima *et al.*, 1993; Park, 2000). The washed muscle is then refined, which removes any remaining bone pieces, skin, scales and connective tissue. The material is then dewatered two or three times by centrifugation, screening or pressing. Dewatering is necessary because during the process water is absorbed (approximately 100% increase) due to repulsion of negatively charged proteins in the washed mince (which is at pH 6.4 to 7.0). The water reduces the repulsion by separating the proteins. Addition of salt (0.1 to 0.3% NaCl or a combination of NaCl and $CaCl_2$) to the wash water further reduces the repulsive forces by shielding negative charges which allows the proteins to be in closer contact with each other, thus expelling water and reducing the tendency of the tissue to absorb water.

Since surimi is generally frozen after dewatering, it is important to protect the functional properties of the product during storage. By adding cryoprotectants to the washed refined material prior to freezing, protein denaturation and aggregation are reduced, which would otherwise result in reduced gelation ability of the proteins (Park and Lanier, 2000). The most common cryoprotectants used in the surimi industry are sorbitol and sucrose at ~8–9%, along with a 1:1 mixture of sodium tripolyphosphate, and tetrasodium pyrophosphate at ~0.2–0.3%. These

compounds are uniformly distributed throughout the surimi by using a silent cutter (Park and Morrissey, 2000). Prior to freezing, proteolytic enzyme inhibitors are sometimes added along with cryoprotectants to prevent proteolytic degradation of proteins during heating. For example in Pacific whiting surimi manufacturing enzyme inhibitors have to be added as well as the application of a very rapid heating rate to minimize proteolytic degradation of muscle proteins (Klesk *et al.*, 2000; Park and Lanier, 2000). Surimi is then frozen in blocks or in chips or chunks (Park and Morrissey, 2000).

High quality surimi has generally only been produced from lean white fleshed fish such as Alaska pollock. However, much effort has been put into how to make good surimi from dark fleshed underutilized species as well as by-products. Most of these attempts have led to products with poor gelation properties, in part due to the low pH of the muscle of these species and different protein isomers in dark vs. white muscle. Considerable color and lipid stability problems are also encountered with surimi from dark muscle species and by-products due to the high amount of lipids, pro-oxidants and pigments (Okada, 1980; Hultin and Kelleher, 2000). Studies have shown that oxidative problems can be reduced with proper processing techniques. For example, having the wash-water alkaline or mincing fish tissue underwater (preferably in an alkaline solution) may reduce rancidity and improve gel strength (Hultin and Kelleher, 2000). Also applying antioxidants early on during processing may significantly increase gel strength and oxidative stability (Kelleher *et al.*, 1994). Many researchers have also proposed ways to remove the dark muscle of these species and process only the light muscle (e.g., Langmyhr *et al.*, 1988; Shimizu *et al.*, 1992; Spencer and Tung, 1994). In some cases this has led to high quality surimi, comparable to that from Alaska pollock, which is the industry standard (Ishikawa *et al.*, 1977; Suzuki and Watabe, 1987). However, this practice becomes very expensive as it requires filleting and then deep skinning the fillets to remove the dark muscle, which is highly unfeasible for small pelagic fish species due to prohibitive cost of labor. Also, the more dark muscle is removed so is some light muscle and thus yield drops. In addition, utilizing only the light muscle of fish will lead to substantial loss of protein. Even just obtaining the fillets from these species can be too cost prohibitive. Also, even if fish can be headed and gutted, some tissues such as the black skin layer on their belly flaps and the kidney tissue along the backbone make processing whole fish far more difficult than just the muscle (Hultin and Kelleher, 2000). The requirement to use very fresh, preferably lean white fleshed fish fillets and the high labor needed for conventional surimi processing, therefore makes it a relatively expensive process. The large quantities of water used also increase the cost of this process.

7.5 Fish protein isolates: pH-shift processing

Two recent processes, developed by Dr Herbert O. Hultin and coworkers (Hultin and Kelleher, 1999; Hultin *et al.*, 2004, 2005), involving acid and alkaline

solubilization and isoelectric precipitation of muscle protein are specifically designed to recover highly functional, stable proteins from low value underutilized fish and by-products. These new processes have shown great promise for both cold and warm water fish species and are currently being commercialized for several species. Fish proteins can be solubilized without the addition of salt at very low and high pH values. The high and low pH values give the muscle proteins a large net charge, causing them to solubilize. At the same time as the proteins solubilize at extremes of pH, the cellular lipid membrane encasing the myofibrillar proteins is disrupted causing a dramatic drop in solution viscosity (Kristinsson, 2002; Kelleher *et al.*, 2004). This allows new approaches to be taken to economically recover fish muscle proteins to produce functional protein isolates from fish sources of low value (Hultin and Kelleher, 1999; Hultin *et al.*, 2004). The process (Fig. 7.3) involves subjecting a diluted slurry (5–10 fold dilution) of finely homogenized muscle tissue to either a very low pH (~2.5–3) or very high pH (~10.8–11.2) at low temperatures. The solubilization of the muscle proteins, cellular membrane disruption and dramatic

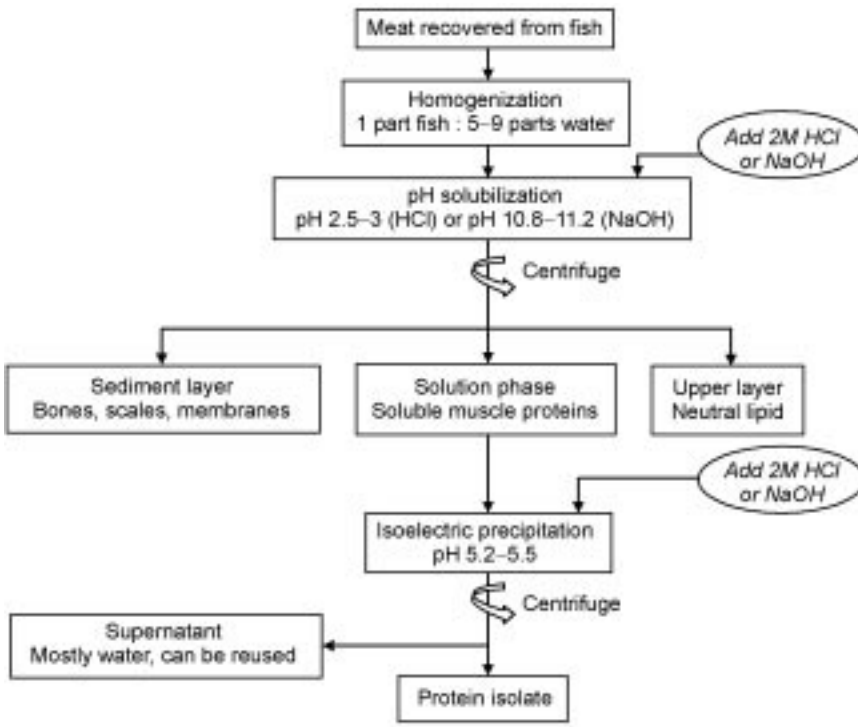

Fig. 7.3 Schematic representation of the acid and alkaline processes used in the production of functional fish protein isolates. The process involves solubilizing muscle proteins at low or high pH, separating them from undesirable muscle components via centrifugation and recovery of the proteins of interest by isoelectric precipitation. The final protein isolate can then be used directly, or stabilized with cryoprotectants and frozen until used. (Adapted from Kelleher and Hultin, 2000.)

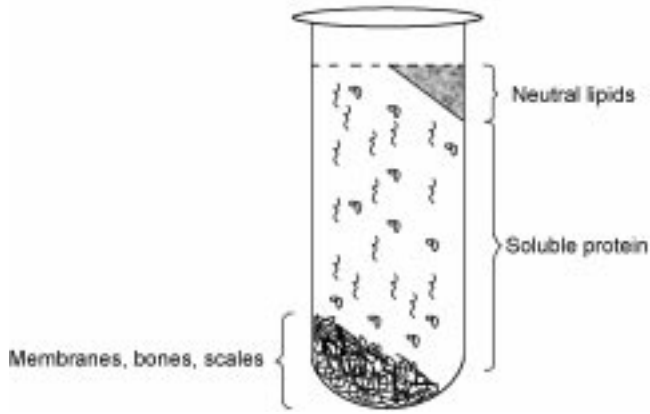

Fig. 7.4 The three phases that develop after the first centrifugation step at low or high pH. The soluble protein is collected and sedimented by isoelectric precipitation aided by centrifugation to prepare the protein isolate.

drop in viscosity enable the cellular lipid membranes to be separated from the soluble proteins by centrifugation (Kelleher and Hultin, 2000), at the same time removing solids such as bones, scales and neutral fat, which are not desired in the final product (Fig. 7.4). The soluble proteins are then collected and recovered by adjusting the pH to *ca.* 5.2–5.5, the isoelectric pH of most muscle proteins (primarily myofibrillar proteins), causing them to aggregate and precipitate to give a protein pellet, i.e. the protein isolate.

There are several significant benefits of these processes compared to harsh chemical extraction and hydrolysis processes and surimi processing. Whole fish with skin and bones, and fatty fish can potentially be utilized in the acid- and alkali-aided processes because proteins are selectively separated and recovered from undesirable muscle components. This is not feasible using typical surimi processing without greatly negatively affecting the recoveries and quality (Hultin, 2002). In the acid- and alkali-aided processes protein recoveries are normally significantly higher compared to many other recovery processes. Significant amounts of proteins are often lost during the production of FPC as well as hydrolysis. Conventional surimi processing leads to the loss of almost all sarcoplasmic protein during the washing steps, upwards of 35% of the total protein. Multiple washings furthermore lead to myofibrillar protein solubilization and consequently some loss of these proteins as well (Lin and Park, 1996). The loss of these proteins during conventional surimi processing is responsible for the significant decrease in yield. The sarcoplasmic proteins are, however, largely recovered in the acid- and alkali-aided processes, thus substantially increasing yield. As a testament to this, using Pacific whiting fillets as the starting material, a conventional three-washing cycle surimi processing yielded only 40% recovery, compared with 60% recovery using acid-aided processing (Choi and Park, 2002). Kristinsson and coworkers (2005) published data on catfish muscle, and found that the acid- and alkali-aided processes had

Table 7.1 Comparison of protein recovery and lipid reduction for the acid-aided, alkali-aided and surimi processes (adapted from Kristinsson *et al.*, 2005)

	Protein recovery	Lipid reduction (neutral and polar)
Alkali-aided process	$70.3 \pm 2.9\%^{b}$	$88.6 \pm 2.8\%^{c}$
Acid-aided process	$71.5 \pm 4.5\%^{b}$	$85.4 \pm 2.0\%^{b}$
Surimi processing	$62.3 \pm 3.1\%^{a}$	$58.3 \pm 7.8\%^{a}$

Means within one column having different superscript letters are significantly different ($p < 0.05$).

significantly higher protein recovery than a lab-scale surimi process (Table 7.1). Similar recoveries were recorded for herring light muscle; 74% for acid-aided process and 68% for alkali-aided process (Undeland *et al.*, 2002). The protein recovery can be further increased when the first centrifugation step is omitted (Kristinsson *et al.*, 2005). This is possible for certain raw materials which are stable towards lipid oxidation, and can bring the protein recovery close to 90%. The bottom sediment from the first centrifugation can also be reprocessed to increase the level of protein extracted (Kristinsson and Demir, 2003). The isoelectric precipitation step in the acid- and alkali-aided process also aids in the higher protein yields as compared to conventional surimi processing. This is due to the protein having a zero net charge at their pI, thus leading to aggregation and precipitation of the proteins. Surimi processing, on the other hand, does not involve reducing the native pH, resulting in a moderate negative charge on the protein molecules which gives them more solubility and thus more proteins are leached out during washing.

Generally, the acid-aided process has resulted in slightly higher protein yields compared to the alkali-aided process with a few exceptions (e.g., tilapia). Studies by Kristinsson and coworkers (2005) have demonstrated that more of the sarcoplasmic proteins are recovered for the acid-aided process compared to the alkali-aided process, due to more aggregation at pH 5.5 as a consequence of a more improperly refolded protein structure. Many of the sarcoplasmic proteins are not greatly affected by the high pH in the alkaline process, and are thus partly or fully native when readjusted to pH 5.5. That pH is away from the isoelectric point of many sarcoplasmic proteins, and thus they don't ready aggregate and co-precipitate with the aggregated myofibrillar proteins. The higher recovery of sarcoplasmic proteins for the acid process, however, means that more heme proteins are recovered with the isolate, which can have negative consequences on color, odor and eventually flavor. More retention on sarcoplasmic proteins in the isolate does, on the other hand, translate to fewer pollution problems since the processing water has a low biological oxygen demand due to its relatively low protein content. This is not the case for surimi processing.

A major advantage of the acid and alkali isolation processes is that undesirable compounds, like skin, bones, microorganisms, cholesterol, membrane lipids, and other contaminating materials are removed during the first centrifug-

ation step (Hultin and Kelleher, 2000). Work on catfish has demonstrated that both processes lead to a significant reduction in aerobic bacteria and also a longer bacterial shelf life compared to surimi from the same starting raw material (Kristinsson, 2004). This work demonstrated that bacteria are both killed/injured by the high and low pH and are also removed during the first centrifugation step in the bottom sediment. The processes also have the potential to remove lipid soluble toxins such as mercury and PCBs, and along with the reduction in bacteria give a safer fish protein product. Removal of most lipid components in the acid and isolation procedures can lead to greater oxidation stability and decreased off-odor development as compared with conventional surimi where membrane lipids mostly remain (Hultin and Kelleher, 2000). The great reduction in lipids is a key step in this process, since many materials of interest are rich in triacylglycerols (neutral storage lipids) and in particular membrane phospholipids due to high amounts of mitochondria in dark muscle (Hultin, 1994). The substantial absence of membranes and neutral lipids in the protein isolate clearly distinguishes the acid and alkali processes from presently available processes. Cholesterol is also reduced in the process due to the removal of membranes (Mireles Dewitt *et al.*, 2002). Due to the higher unsaturation of phospholipids vs. neutral lipids and their greater surface area exposed to the cell aqueous phase, they are known to be the main substrate for oxidative reactions in muscle foods (Shewfelt, 1981; Gandemer, 1999). Their removal is therefore expected to greatly enhance the oxidative stability of the final protein isolate. This is not achieved in conventional processes unless organic solvents are used, which destroys protein functional properties.

Kristinsson and Demir (2003) studied four different species and reported total lipid reduction of 58.3, 72.1, 10.4 and 16.7% for surimi processing (catfish, Spanish mackerel, mullet and croaker, respectively), 85.4, 76.9, 58, 38.1% for the acid-aided process, and 88.6, 79.1, 81.4 and 68.4% for the alkali-aided process. Undeland and coworkers (2002) reported ~70% reduction in neutral lipids and ~50% reduction in membrane phospholipids for herring white muscle using both acid and alkali-aided processes. Undeland and coworkers (2005) later reported that including centrifugation in the solubilization step to remove membranes led to about 50% less development of secondary oxidation products (as measured by TBARS analysis). Kristinsson (2004) also demonstrated that catfish protein isolates have lower TBARS values on storage when centrifugation is included in the solubilization step, compared to skipping the centrifugation. Kristinsson and Demir (2003) showed that the alkali-aided process gives a more oxidatively stable protein isolate at pH 5.5 than the acid-aided process, and in many cases is more stable than surimi (Fig. 7.5). Substantial lipid oxidation was seen during the acid-aided process. Petty and Kristinsson (2004) investigated oxidation at low and high pH in detail for Spanish mackerel muscle homogenates and showed extensive oxidation development at low pH but almost no development at high pH. When the homogenate was adjusted from low and high pH to pH 5.5 or 7, the isolates from the acid-aided process oxidized significantly more than those form the alkali-aided process. The same was seen

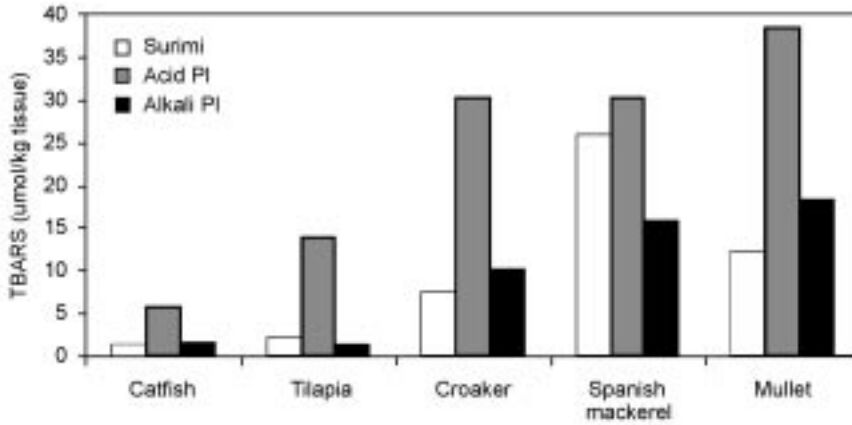

Fig. 7.5 Levels of oxidation for surimi and acid and alkali isolates at day 3 of refrigerated storage (4ºC) as assessed by TBARS (secondary oxidation products). Acid isolates were made using pH 2.5 as the solubilization pH, while alkali isolates were made using pH 11. Isolates were recovered at pH 5.5 and stored at 4ºC. Surimi was made by by washing ground muscle in 3 volumes of water 3 times, with the last wash containing 0.3% NaCl to aid in dewatering. The pH of the surimi was not adjusted and was from pH 6.5–6.6 for all species. Surimi was also stored at 4ºC.

in a model system with trout hemoglobin and washed cod muscle, and it appears that the hemoglobin (one of the main catalysts/mediators of oxidation in fish muscle) was effectively stabilized at high pH but became highly pro-oxidative at low pH (Kristinsson and Hultin, 2004). Undeland and coworkers (2005) also reported extensive oxidation for herring isolates made with the acid-aided process. This oxidation could be effectively reduced and delayed by employing proper antioxidant treatments, such as erythorbate, EDTA and sodium tripolyphoshpate (Undeland *et al.*, 2005). Undeland and coworkers (2005) demonstrated that if antioxidative treatments (metal reducing agents and metal chelators) are incorporated early on in the extraction process, e.g. during homogenization, oxidative stability of the isolate can be improved. Speed of processing at the extreme pH appears to be important. Lipid oxidation can be somewhat reduced if the system is at low pH for a very brief time before being adjusted to pH 5.5 (Hultin, 2004; Petty and Kristinsson, 2004).

Heme proteins are more effectively removed with the alkali-aided process compared to surimi processing, resulting in a product that is whiter and more stable to lipid oxidation. Heme proteins are also protected from denaturation and autoxidation during high pH treatment at low temperature (Kristinsson, 2002). The acid aided process, however, leads to the denaturation of heme proteins and thus co-precipitation with muscle proteins when they are adjusted to pH 5.5 (Kristinsson and Hultin, 2004; Kristinsson *et al.*, 2005). This leads to an isolate with darker color and more oxidative problems (Kristinsson and Demir, 2003). Choi and Park (2002) reported that whiteness was lower in acid-treated Pacific whiting isolates as compared with conventional surimi. This lower whiteness of

Table 7.2 Color of protein isolates and surimi according to Hunter L*, a* and b* values (adapted from Kristinsson and Demir, 2003)

Sample	L*	a*	b*
Catfish alkali PI	75.0 ± 0.7	−3.0 ± 0.2	0.2 ± 0.4
Catfish acid PI	73.8 ± 0.4	−3.6 ± 0.2	5.7 ± 0.3
Catfish surimi	70.4 ± 1.1	−0.9 ± 0.2	0.7 ± 0.4
Croaker alkali PI	67.6 ± 0.4	−2.1 ± 0.2	4.2 ± 0.4
Croaker acid PI	69.8 ± 0.7	−2.0 ± 0.3	7.8 ± 0.4
Croaker surimi	64.7 ± 2.4	0.2 ± 0.1	6.4 ± 0.9

the acid isolates was attributed to higher b* values, indicating a more yellow appearance. Kristinsson and Demir (2003) demonstrated that color was generally good for isolates made from several warm water species using the acid- and alkali-aided processes as compared to surimi from the same species. Table 7.2 shows the color for catfish and croaker isolates and surimi. Isolates had higher L* values and whiteness than surimi, however the acid isolates had higher yellowness, possibly since more denatured heme proteins are found in these isolates (Kristinsson and Demir, 2003).

It has been found that functional properties are retained, decreased (in few cases for the acid process) or often significantly improved (most notably for the alkali process) using the pH-shift processes to recover fish proteins. The main functional property of extracted fish proteins is their ability to form strong and elastic gels with high water-holding capacity. Research shows that the ability of isolates to form gels varies, depending on species and conditions used to make the isolate. Hultin and Kelleher (2000) reported that acid-aided isolates made from Atlantic cod and mackerel produces good gels. Later it was found that Pacific whiting surimi from a 3-cycle washing method made stronger gels than gels from the acid-aided process (Choi and Park, 2002). Work by Kristinsson (2004) has demonstrated that the acid-aided process in some cases does form better gels than surimi but in some cases worse. All studies by this group, however, clearly show that the alkali-aided process produces superior gels over both the acid-aided process and the surimi process. For example, Ingadottir and Kristinsson (2004, 2005) reported significantly higher gel strength and elasticity for tilapia isolates made with the alkali-aided process compared to the acid-aided process and surimi (Fig. 7.6). Davenport and Kristinsson (2004) did also report using oscillatory rheology and torsion testing that catfish protein isolates from the acid-aided process have significantly lower gel-forming ability than isolates from the alkali-aided process, and hypothesize that this could be due to some very different effects on protein structure at acid vs. alkali pH. Another study by Yongsawatdigul and Park (2001), demonstrated that rockfish protein isolates produced from the alkali-aided process had better gel-forming ability as compared to the acid-aided and conventional surimi processes. The lower performance of the isolates from the acid-aided process could be due to

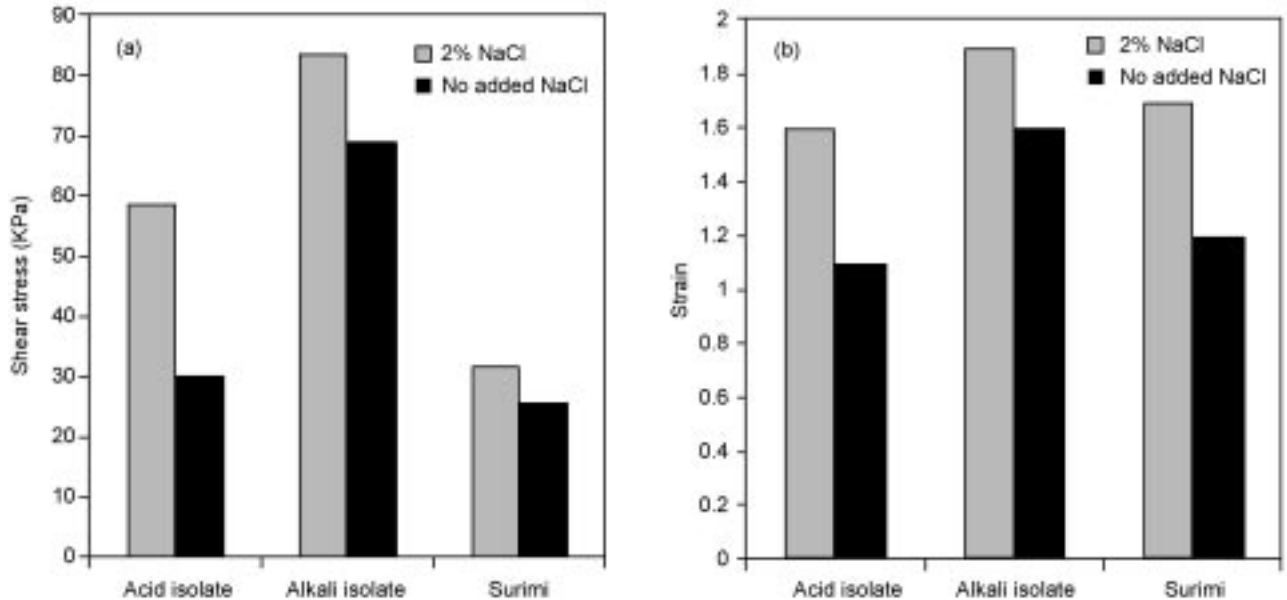

Fig. 7.6 Shear stress (kPa) and strain values of gels produced from tilapia acid protein isolate, alkali protein isolate and surimi. The acid isolates were made by using pH 2.9 as the solubilization pH, while the alkali isolates were made using pH 11. Isolates were recovered at pH 5.5. The surimi was made by washing ground muscle in 3 volumes of water 3 times, with the last wash containing 0.3% NaCl to aid in dewatering. A protein paste was made with or without the addition of 2% NaCl, and adjusted to pH 7.2. No cryoprotectants were added. Pastes were cooked in steel tubes at 80ºC for 30 min to form a gel. The gels were stored in a cold room at 4ºC for 48 hours prior to testing with a Torsion Gelometer. (Adapted from Ingadottir and Kristinsson, 2005.)

proteolysis which has been seen during the low pH solubilization step (Choi and Park, 2002; Undeland *et al.*, 2002; Ingadottir and Kristinsson, 2004).

Proteolysis is a major problem for any muscle protein extraction process as it will lead to adverse effects on protein functionality, particularly gelation and water-binding. Both Ingadottir and Kristinsson (2005) and Undeland *et al.* (2002) found proteolysis of myosin at low pH. Kristinsson and Demir (2003) reported the same findings for Spanish mackerel. For some species, proteolysis can also occur at the recovery pH, i.e. pH 5.5. Choi and Park (2002) showed that cathepsin B and L activity was higher in an acid-treated Pacific whiting as compared with a 3-cycle washed surimi, leading to poorer gel-forming ability for the isolate. The differences between the cathepsin activity levels were due to cathepsin B and H removal during repeat washing and pH 5.5 being the optimum pH for cathepsin L activity (An *et al.* 1994; Choi and Park, 2002). However, cathepsin H was removed from the surimi and inactivated in the acid isolate. Therefore, this enzyme did not contribute to decreased gel-forming ability in either sample. Some species have, however, not demonstrated any proteolysis at low pH, e.g. cod (Hultin and Kelleher, 2000) and catfish (Kristinsson *et al.*, 2005). Cod isolate made with the acid-aided process makes a good gel, while the catfish isolate made with the acid-aided process makes a poorer gel than isolates from the alkali-aided process. The difference has been linked to how the muscle proteins respond differently to changes in pH. Davenport and Kristinsson (2003) reported that catfish myosin adjusted to a low pH (2.5) and then readjusted to pH 7 had significantly less gel-forming ability compared to myosin adjusted to high pH (11) and then readjusted to pH 7. The difference was not attributed to proteolysis, but rather some changes within the protein, which are yet to be fully understood. Previous work with cod myosin demonstrated that acid and alkali treated myosin performed about the same as untreated myosin (Kristinsson and Hultin, 2003). This demonstrates that proteins from different species do respond differently to these processes.

The theory has long been that the sarcoplasmic proteins interfere with gel formation, possibly by binding to the myofibrillar proteins on heating (Okada, 1980; Shimizu *et al.*, 1992; Park *et al.*, 1997). This is one of the arguments why these proteins are removed in surimi. However, some recent studies have challenged this belief and have shown that gel strength is either equal or, in fact, enhanced by the presence of the sarcoplasmic proteins (Morioka and Shimizu, 1990; Ko and Hwang, 1995). The presence of the sarcoplasmic proteins in the isolates from the acid and alkali process does not appear to negatively impact the gel strength of the final product (Hultin and Kelleher, 1999) but gel mechanism may be different as these proteins have been acid or alkali denatured. This may, however, be species dependent. The acid-aided process does recover more of the sarcoplasmic proteins, and as has been discussed above, some species produce acid isolates of poor functionality, which could be linked to the higher levels of denatured sarcoplasmic proteins. Kristinsson and Crynen (2003) studied the gel-forming ability of myofibrillar and sarcoplasmic proteins from catfish, indivi-

dually and in combination. The results indicated that sarcoplasmic proteins subjected to a low and high pH and added to myofibrillar proteins subjected to the same treatment improve the gel-forming ability of the overall system compared to myofibrillar proteins alone. The results suggested that adverse changes in the myofibrillar proteins at low pH (pH 2.5) and positive change in the myofibrillar proteins at high pH (pH 11) may explain the difference between the performance of the acid and alkali isolates, rather than changes in the sarcoplasmic proteins. This is being investigated in more detail.

It is a commonly held view that denaturing fish muscle proteins has a detrimental impact on their functional properties (Konno *et al.*, 1997; Visessanguan and An, 2000). As the muscle proteins experience very low or high pH in the acid and alkaline processes, one might assume that they lose their functionality since they are partly denatured. Preliminary work by Kristinsson (unpublished data) on the cellular organization of the muscle cell using phase contrast microscopy showed that as pH is either lowered or increased, the contractile element within the cell is being distorted as its protein constituents are being progressively more charged, repelling each other and eventually they become solubilized and any remnants of the muscle cell are lost. Upon pH readjustment to pH 5.5 the cell structure is clearly not recovered and an aggregate of partially denatured muscle proteins is observed. These findings are supported by recent electron microscope data by Wright and Lanier (2005). Studies on the molecular level with myosin have demonstrated that the protein subunit assembly and tertiary structure are greatly affected by the low and high pH, and are not reversibly fully refolded or reassembled on pH readjustment to neutrality (Kristinsson and Hultin, 2003). Furthermore, the ATPase activity of myosin is almost completely lost on acid or alkali treatment (Kristinsson and Hultin, 2003). Other workers have also shown that isolates made with the process have little or no ATPase activity, yet they have good gel-forming ability. Studies frequently report on the positive relationship between functional ATPase activity and functionality of muscle proteins (Katoh *et al.*, 1979; Ooizumi *et al.*, 1981; Konno *et al.*, 1997). Interestingly, even when all ATPase activity is lost in the isolate, some of them have improved gelling capability compared to proteins still with high ATPase activity. These findings suggest that a native structure is not required for good gel-forming ability, and a partly pH denatured protein may, in fact, be better suited to form quality gels, perhaps through different mechanisms than native proteins.

7.6 Other processes using low or high pH

Only a handful of other workers have described processes for fish proteins that utilize high or low pH. These processes, however, differ considerably in nature to the one described above and the end uses are quite different. Cuq and coworkers (1995) reported on the acid solubilization of fish muscle proteins at pH 3 using aqueous acetic acid for the purpose of producing edible packaging

films. A process was reported by Shahidi and Venugopal (1993) where minced Atlantic mackerel, herring or capelin is homogenized in aqueous liquids, including acetic acid at pH 3.5. Venugopal and Shahidi (1994) later reported a process where Atlantic mackerel is suspended in water and acetic acid at a pH of 3.5. The use of acetic acid in these processes, however, in many cases increases viscosity of these fish protein suspensions and in some cases reduces it insufficiently so that cellular lipid membranes cannot be separated from the fish proteins. The volatile acetic acid also potentially leads to a strong odor to the final material which may limit their use as food products. Some of the above processes also involved washing steps which remove the water soluble sarcoplasmic muscle proteins, which are retained in the acid and alkaline processes described previously.

Alkaline pH has also been used in the processing of fish muscle and muscle from other sources. One common use of high pH is to recover protein from deboned meat (McCurdy *et al.*, 1986; Opiacha *et al.*, 1994), not, however, involving separation of membrane lipids from the alkali solubilized protein. Alkaline conditions have been used previously in the manufacture of surimi from fish, where alkali or compounds with buffering capacity at high pH are added to the wash water to increase pH. The wash water pH in these processes is, however, considerably lower than the pH used in the alkaline process employed in this proposal. The increase of wash water pH reportedly yields a product with improved gelling abilities, brighter color and lower lipid content (Shimizu *et al.*, 1992; Jiang *et al.*, 1998; Hultin and Kelleher, 1999). On the other hand, yield drops considerably in these processes, since presumably increased protein solubility as pH is increased would increase the amount of proteins removed in the washing steps. For example, it has been reported that processing surimi from mackerel light muscle using alkaline wash water led to only 40% protein recovery (Hultin and Kelleher, 2000).

7.7 Future trends

To meet the increasing demand for quality fish proteins and products containing fish proteins, it is of great importance to utilize our raw materials more responsibly as well as to find new resources of fish to utilize. Processing methods which can employ inexpensive raw material and whole fish instead of fillets would create a significant economic advantage to the fish protein ingredient industry, as new sources of fish unsuitable for conventional processes could enter the market, and production could be increased to meet the world demand. This chapter has reviewed some of the chemical processes which can be employed to recover fish proteins, as well as lipids. From looking at research and industrial applications of these processes, the pH-shift processing appears to have the most promise on by-products and underutilized species. To increase the success of these processes and their products it is essential to put more research efforts into the commercial applications of these proteins as food ingredients or

as food products. More research should be focused on the utilization of extracted fish proteins for human consumption rather than animal consumption, although the latter cannot be overlooked. One very promising food application being studied is the use of isolated fish proteins as water-binders in seafood products. It has been found that these proteins can effectively compete with and outperform phosphates as water-binders. This could have a significant meaning for the seafood processing industry. Future research efforts should also be directed towards ways to effectively stabilize the proteins against functional changes as well as finding efficient and economic ways to stabilize the isolated proteins against oxidative changes (e.g., lipid oxidation during processing and in the final isolate). The future for functional fish protein ingredients is promising and with the right mindset from industry, government and academia great progress can be made in the near future.

7.8 References and further reading

AN, H., WEERASINGHE, V., SEYMOUR, T. A. and MORRISSEY, M. T. (1994), Cathepsin degradation of Pacific whiting surimi proteins. *J. Food Sci.,* **59**, 1013–1033.

ANONYMOUS (2001), Frozen surimi market booms in Europe, sparking new production to meet demand. *Quick Frozen Foods Int.,* **6**, 22.

BLENFORD, D. E. (1994), Protein hydrolysates; functionalities and uses in nutritional products, *Int. Food Ingr.,* **3**, 45.

CHEFTEL, C., AHERN, M., WANG, D. I. C. and TANNENBAUM, S. R. (1971), Enzymatic solubilization of fish protein concentrate: Batch studies applicable to continuous enzyme recycling processes, *J. Agr. Food Chem.,* **19**, 155.

CHOI, Y. J. and PARK, J. W. (2002), Acid-aided protein recovery from enzyme-rich pacific whiting. *J. Food Sci*., **67**, 2962–2967.

CUQ, B., AYMARD, C., CUQ, J. L. and GUILBERT, S. (1995), Edible packaging films based on fish myofibrillar proteins: Formulation and functional properties. *J. Food Sci.,* **60**, 1369–1374.

CUSHMAN JR., J. H. (1998), Cuts sought in wasteful fish kills. *New York Times – Science Times*, January 13, 2.

DAVENPORT, M. and KRISTINSSON, H. G. (2003), Low and high pH treatments induce a molten globular structure in myosin which improves its gelation properties. IFT Annual Meeting, Chicago, IL, Abstract 42-9.

DAVENPORT, M. and KRISTINSSON, H. G. (2004), Effect of different acid and alkali-treatments on the molecular and functional properties of catfish muscle proteins. IFT Annual Meeting, July 12–16, Las Vegas, NV. Abstract 49G-14.

DAVENPORT, M. and KRISTINSSON, H. G. (2005), Effect of cold storage and freezing of ground catfish muscle prior to acid or alkali-aided isolation of muscle proteins. IFT Annual Meeting, July 15–20, New Orleans, LA. Abstract 89B-33.

DUBROW, D. L., KRAMER, A. and McPHEE, A. D. (1973), Effects of temperature on lipid extraction and functional properties of fish protein concentrate (FPC). *J. Food Sci.,* **38**, 1012.

FAO (2000), The State of World Fisheries and Aquaculture; FAO Fisheries Department, Food and Agricultural Organization of the United Nations: Rome, Italy.

GANDEMER, G. (1999), Lipids and meat quality: lipolysis, oxidation, Maillard reaction and flavour. *Sci. Aliment.* **19**, 439–458.

GWENN, S. E. (1992), Development of Surimi Technology in the United States. In T. C. Lanier and C. M. Lee (eds), *Surimi Technology*, New York, Marcel Dekker, Inc., 23–39.

HALE, M. B. (1972), Making fish protein concentrate by enzymatic hydrolysis, NOAA Technical Report NMFS SSRF-675, US Department of Commerce, Seattle, WA, 1–31.

HERMANSSON, A. M., SIVIK, B. and SKJOLDEBRAND, C. (1971), Factors affecting solubility, foaming and swelling of fish protein concentrate, *Lebensm. Wiss. Technol.,* **4**, 201.

HOYLE, N. and MERRITT, J. H. (1994), Quality of fish protein hydrolysates from herring (*Clupea harengus*), *J. Food Sci.,* **59**, 76.

HULTIN, H. O. (1994), Oxidation of lipids in seafoods. In F. Shahidi and J. R. Botta, *Seafoods: Chemistry, Processing Technology and Quality*, Glasgow, Blackie Academic, 49–74.

HULTIN, H. O. (2002), Recent Advances in Surimi Technology. In M. Fingerman and R. Nagabhushanam, *Recent Advances in Marine Biotechnology*, Enfield, New Hampshire, Science Publisher, Inc., 241–251.

HULTIN, H. O. (2004), Controlling lipid oxidation during processing at low and high pH. In symposium: Fish Protein Recovery Using pH Shifts. IFT Annual Meeting, Las Vegas, NV, July 12–16, 2004.

HULTIN, H. O. and KELLEHER, S. D. (1999), Process for isolating a protein composition from a muscle source and protein composition. US Patent No. 6,005,073.

HULTIN, H. O. and KELLEHER, S. D. (2000), Surimi processing from dark muscle fish. In J. W. Park *Surimi and Surimi Seafood*, New York, Marcel Dekker, 59–77.

HULTIN, H. O., KELLEHER, S. D., FENG, Y., KRISTINSSON, H. G., RICHARDS, M. P., UNDELAND, I. A. and KE, S. (2004), US Patent Application No. 10/363,612. 'High Efficiency Alkaline Protein Extraction'.

HULTIN, H. O., KRISTINSSON, H. G., LANIER, T. C. and PARK, J. W. (2005), Process for recovery of functional proteins by pH shifts. In J. W. Park, *Surimi and Surimi Seafood*, 2nd edn, New York, Marcel Dekker, 107–139.

INGADOTTIR, B. and KRISTINSSON, H. G. (2004), Effects of low and high pH treatments on solubility and viscosity of tilapia light muscle homogenates. IFT Annual Meeting, Las Vegas, NV, July 12–16, 2004, Abstract 17E-20.

INGADOTTIR, B. and KRISTINSSON, H. G. (2005), Recovery and gel forming ability of proteins recovered from tilapia muscle with acid and alkali aided solubilization and precipitation. IFT Annual Meeting, July 15–20, New Orleans, LA. Abstract 89B-31.

ISHIKAWA, S., NAKAMURA, K. and FUJII, Y. (1977), Test program to manufacture sardine-based products and frozen surimi. I. Effects of freshness of material and fish dressing methods. *Tokai Fisheries Research Agency Japan*, **20**, 59–66.

JIANG, S-H., HO, M-L., JIANG, S-H., LO, L. and CHEN, H-C. (1998), Color and quality of mackerel surimi as affected by alkaline washing and ozonation. *J. Food Sci.*, **63**, 652–655.

KATOH, N., NOZAKI, H., KOMATSU, K. and ARAI, K. (1979), A new method for evaluation of the quality of frozen surimi from Alaska pollack. Relationship between myofibrillar ATPase acitivity and Kamaboko forming ability of frozen surimi. *Nippon Suisan Gakkaishi*, **45**, 1027–1032.

KELLEHER, S. D. and HULTIN, H. O. (2000), Functional chicken muscle protein isolates prepared using low ionic strength, acid solubilization/precipitation. *Rec. Meat. Conf. Proc.*, **53**, 76–81.

KELLEHER, S. D., HULTIN, H. O. and WILHELM, K. A. (1994) Stability of mackerel surimi prepared under lipid-stabilizing processing conditions. *J. Food Sci.*, **59**, 269–271.

KELLEHER, S. D., FENG, Y., KRISTINSSON, H., HULTIN, H. O. and McCLEMENTS, D. J. (2004), Functional fish protein isolates prepared using low ionic strength, acid solubilization/precipitation. In: *More Efficient Utilization of Fish and Fisheries Products*. New York, Elsevier Science, 407–414.

KINSELLA, J. E. (1976), Functional properties of proteins in foods: A survey, *Crit. Rev. Food Sci. and Nutr.*, **8**, 219.

KLESK, K., YONGSAWATDIGUL, J., PARK, J. W., VIRATCHAKUL, S. and VIRULHAKUL, P. (2000), Gel forming ability of tropical tilapia surimi as compared with Alaska pollock and Pacific whiting surimi. *J. Aquat. Food Prod. Technol.*, **9**, 91–104.

KO, W-C. and HWANG, M-S. (1995), Contribution of milkfish sarcoplasmic proteins to gel formation of fish meat. *Nippon Suisan Gakkaishi*, **61**, 75–78.

KONNO, K., YAMANODERA, K.-I. and KIUCHI, H. (1997), Solubilization of fish muscle myosin by sorbitol. *J. Food Sci.*, **62**, 980–984.

KRISTINSSON, H. G. (2002), Conformational and functional changes of hemoglobin and myosin induced by pH: Functional role in fish quality. PhD dissertation. University of Massachusetts at Amherst.

KRISTINSSON, H. G. (2004), Using high and low pH processing to produce functional protein ingredients from warm water fish species. In symposium: Fish Protein Recovery Using pH Shifts. IFT Annual Meeting, Las Vegas, NV, July 12–16, 2004.

KRISTINSSON, H. G. and CRYNEN, S. (2003), The effect of acid and alkali treatments on the gelation properties of sarcoplasmic and myofibrillar proteins from channel catfish. IFT Annual Meeting, Chicago, IL, Abstract 87-3.

KRISTINSSON, H. G. and DEMIR, N. (2003), Functional protein isolates from underutilized tropical and subtropical fish species and byproducts. In P. Bechtel, *Advances in Seafood Byproducts*, Alaska Sea Grant College Program, University of Alaska, Anchorage, 277–298.

KRISTINSSON, H. G. and HULTIN, H. O. (2003), Changes in conformation and subunit assembly of cod myosin at low and high pH and after subsequent refolding. *J. Agric. Food Chem.*, **51**, 7187–7196.

KRISTINSSON, H. G. and HULTIN, H. O. (2004), Changes in trout hemoglobin conformations and solubility after exposure to acid and alkali pH. *J. Agric. Food Chem.*, **52**, 3633–3643.

KRISTINSSON, H. G. and RASCO, B. A. (2000), Fish protein hydrolysates: Production, biochemical and functional properties. *CRC Crit. Rev. Food Sci. Hum. Nutr.*, **40**, 43–81.

KRISTINSSON, H. G. and RASCO, B. A. (2002), Fish protein hydrolysates and their potential use in the food industry. In M. Fingerman and R. Nagabhushanam, *Recent Advances in Marine Biotechnology*, Vol. 7, Enfield, NH, Science Publishers, Inc., 157–181.

KRISTINSSON, H. G., THEODORE, A. E., DEMIR, N. and INGADOTTIR, B. (2005), The recovery of channel catfish muscle proteins using acid and alkali-aided processing vs. surimi processing. *J. Food Sci.*, **70**, C298–C306.

LANGMYHR, E., OPSTVEDT, J., OFSTAD, R. and SORENSEN, N. K. (1988), Potential conversion of North Atlantic fatty species into surimi and surimi. In N. Davis, *Fatty Fish Utilization: Upgrading from Feed to Food,* UNC Sea Grant College Program Publication; Raleigh, NC, 1988.

LIN, D. D. and PARK, J. W. (1996), Extraction of proteins from Pacific whiting mince at various washing conditions. *J. Food Sci.*, **61**, 432–438.

LOFFLER, A. (1986), Proteolytic enzymes: sources and applications, *Food Technol.*, **40**, 63.

MACKIE, I. M. (1974), Proteolytic enzymes in recovery of proteins from fish waste, *Proc. Biochem.*, **12**, 12.

MACKIE, I. M. (1982), Fish protein hydrolysates. *Proc. Biochem.*, **17**, 26–32.

McCURDY, S. M., JELEN, P., FEDEC, P. and WOOD, D. F. (1986), Laboratory and pilot plant scale recovery of protein from mechanically separated chicken residue. *J. Food. Sci.*, **51**, 742–747.

MIRELES DEWITT, C. A., GOMEZ, G. and JAMES, J. M. (2002), Protein extraction from beef heart using acid solubilization. *J. Food Sci.*, **67**, 3335–3341.

MOORJANI, M. N., NAIR, R. B. and LAHIRY, N. L. (1968), Quality of fish protein concentrate prepared by direct extraction of fish with various solvents, *Food Technol.*, **22**, 1557.

MORIOKA, K. and SHIMIZU, Y. (1990), Contribution of sarcoplasmic proteins to gel formation of fish meat. *Nippon Suisan Gakkaishi*, **56**, 929–933.

OHSHIMA, T., USHIO, H. and KOIZUMI, C. (1993), High pressure processing of fish and fish products. *Trends in Food Sci and Technol.*, **4**, 370–375.

OKADA, M. (1980), Utilization of small pelagic species for food. In R. E. Martin. *Proceedings of the Third National Technical Seminar on Mechanical Recovery and Utilization of Fish Flesh*, Washington, DC, National Fisheries Institute, 265–282.

OKADA, S. (1992), History of Surimi Technology. In T. C. Lanier and C. M. Lee, *Surimi Technology,* New York, Marcel Dekker, Inc., 3–20.

OOIZUMI, T., HASHIMOTO, A., OGURA, J. and ARAI, K. (1981), Quantitative aspect for protective effect of sugar and sugar alcohol against denaturation of fish myofibrils. *Nippon Suisan Gakkaishi*, **47**, 901–908.

OPIACHA, J. O., MAST, M. G. and MacNEIL, J. H. (1994), Composition of dehydrated protein extracts from poultry bone residue. *J. Muscle Foods*, **5**, 343–353.

ORLOVA, T. A., NELICHIK, N. N. and FLEIDER, K. A. (1979), Edible protein concentrate from fish raw material, *Rybnoe Khozyaistvo*, **10**, 59.

PARK, J. W. (2000), *Surimi and Surimi Seafood*, New York, Marcel Dekker Inc.

PARK, J. W. and LANIER, T. C. (2000), Processing of Surimi and Surimi Seafoods. In R. E. Martin, *Marine and Freshwater Products Handbook*, Lancaster, PA, Technomic Publishing Company.

PARK J. W. and MORRISSEY M. T. (2000), Manufacture of Surimi from Light Muscle Fish. In J. W. Park, *Surimi and Surimi Seafood*, New York, Marcel Dekker, Inc., 23–54.

PARK, J. W., LIN, T. M. and YONGSAWATDIGUL, J. (1997), New developments in manufacturing of surimi and surimi seafood. *Food Rev. Int.*, **13**, 577–610.

PETTY, H. T. and KRISTINSSON, H. G. (2004) Impact of antioxidants on lipid oxidation during acid and alkali processing of Spanish mackerel. IFT Annual Meeting, Las Vegas, NV, July 12–16, 2004, Abstract 49B-7.

QUAGLIA, G. B. and ORBAN, E. (1987a), Enzymic solubilisation of proteins of sardine (Sardina pilchardus) by commercial proteases, *J. Sci. Food Agric.*, **38**, 263.

QUAGLIA, G. B. and ORBAN, E. (1987b), Influence of the degree of hydrolysis on the solubility of the protein hydrolsyates from sardine (*Sardina pilchardus*), *J. Sci. Food Agric.*, **38**, 271.

SHAHIDI, F. and VENUGOPAL, V. (1993), Notes and digest. *Meat Focus Int.* October, 443–445.

SHEUSTONE, F. S. (1953), Forming of fish protein concentrate. *Food Preserv. Q.*, **13**, 45.

SHEWFELT, R. L. (1981), Fish muscle lipolysis – a review. *J. Food Biochem.*, **5**, 79–100.

SHIMIZU, Y., TOYOHARA, H. and LANIER, T. C. (1992), Surimi production from fatty and dark-fleshed fish species. In T. C. Lanier and C. M. Lee, *Surimi Technology*, New York, Marcel Dekker, 181–207.

SIKORSKI, Z. E. and NACZK, M. (1981), Modification of technological properties of fish protein concentrates, *Crit. Rev. Food Sci. Nutr.*, **4**, 201.

SKANDERBY, M. (1994), Protein hydrolysates: their functionality and applications, *Food Technol. Int. Eur.*, **10**, 141.

SNYDER, D. G. (1967), Bureau of commercial fisheries program, *Food Technol.*, **21**(9), 70.

SPENCER, K. E. and TUNG, M. A. (1994), Surimi processing from fatty fish. In F. Shahidi and J. R. Botta, *Seafoods: Chemistry, Processing Technology and Quality*, London, Blackie Academic and Professional.

SPINELLI, J., KOURY, B. and MILLER, R. (1972), Approaches to the utilization of fish for the preparation of protein isolates; enzymic modifications of myofibrillar fish proteins, *J. Food Sci.*, **37**, 604.

SUZUKI, T. and WATABE, S. (1987), New processing technology of small pelagic fish protein. *Food Rev. Int.*, **2**, 271–307.

TANNENBAUM, S. R., AHERN, M. and BATES, R. P. (1970a), Solubilization of fish protein concentrate. 1. An alkaline process, *Food Tecnol.*, **24**, 604–606.

TANNENBAUM, S. R., AHERN, M. and BATES, R. P. (1970b), Solubilization of fish protein concentrate. 2. Utilization of the alkaline-process product, *Food Tecnol.*, **24**, 607.

THAKAR, P. N., PATEL, J. R. and JOSHI, N. S. (1991), Protein hydrolysates: A review. *Indian J. Dairy Sci.*, **44**, 557–588.

THOMAS, D. and LOFFLER, F. (1994), Improved protein functionalities by enzymatic treatment, *Food Marketing and Technology*, **2**.

UNDELAND, I. A., KELLEHER, S. D. and HULTIN, H. O. (2002), Recovery of functional proteins from herring (*Clupea harengus*) light muscle by an acid or alkaline solubilization process. *J. Agric. Food Chem.*, **50**, 7371–7379.

UNDELAND, I., HALL, G., WENDIN, K., GANGBY, I. and RUTGERSSON, A. (2005), Preventing lipid oxidation during recovery of functional proteins from herring (*Clupea harengus*) fillets by an acid solubilization process. *J. Agric. Food Chem.*, **53**, 5625–5634.

UNIDO, SECTORAL STUDIES BRANCH (1990), Industrial development strategies for fishery systems in developing countries. *Food Rev. Int.*, **6**, 1–13.

VARELTZIS, K., SOULTOS, N., ZETOU, F. and TSIARAS, I. (1990), Proximate composition and quality of a hamburger type product made from minced beef and fish protein concentrate, *Lebensm. Wiss. u. Technol.*, **23**, 112.

VENUGOPAL, V. and SHAHIDI, F. (1994), Thermostable water dispersions of myofibrillar proteins from Atlantic mackerel (*Scomber scombrus*). *J. Food Sci.*, **59**, 265–276.

VENUGOPAL, V., CHAWLA, S. P. and NAIR, P. M. (1996), Spray dried protein powder from threadfin beam: preparation, properties and comparison with FPC type-B, *J. Muscle Foods*, **7**, 55.

VISESSANGUAN, W. and AN, H. (2000), Effects of proteolysis and mechanism of gel weakening in heat-induced gelation of fish myosin. *J. Agric. Food Chem.*, **48**, 1024–1032.

WEBSTER, J. D., LEDWARD, D. A. and LAWRIE, R. A. (1982), Protein hydrolysates from meat industry by-products, *Meat Sci.*, **7**, 147.

WRIGHT, B. J. and LANIER, T. C. (2005), Microscopic evaluation of ultrastructural disintegration and dispersion of myofibrillar proteins as affecting gel formation. IFT Annual Meeting, July 15–20, New Orleans, LA. Abstract 50-8.

XIONG, Y. L. (1997), Structure-function relationships of muscle proteins. In S. Damodaran

and A. Paraf, *Food Proteins and Their Applications*, New York, Marcel Dekker, Inc., 341–392.

YONGSAWATDIGUL, J. and PARK, J. W. (2001), Gelation characteristics of alkaline and acid solubilization of fish muscle, IFT National Meeting, New Orleans, La. June 23–27, 2001, Institute of Food Technologists, Abstract number100-1.