RECOVERY OF SOLIDS FROM SURIMI WASH WATER AND PREPARATION OF A FISH FEED WITH THE RECOVERED SOLIDS

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

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DEPARTMENT OF PROCESSING TECHNOLOGY

COLLEGE OF FISHERIES

PANANGAD, KOCHI

To

My daughter

DECLARATION

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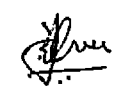

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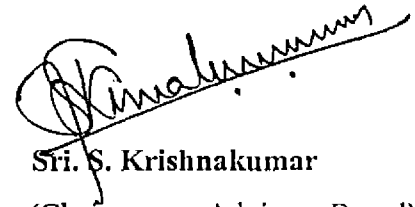

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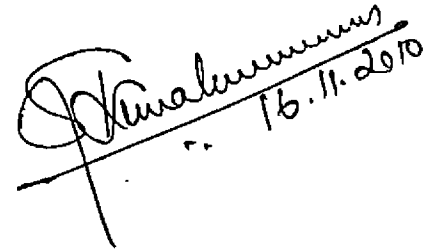

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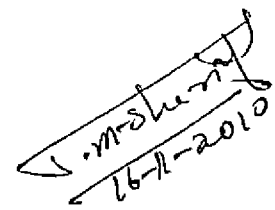

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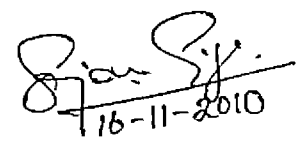

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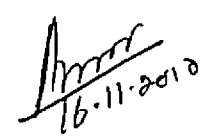

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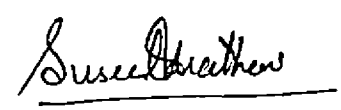

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Jibina M. M.

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INTRODUCTION

1. INTRODUCTION

Surimi technology has its historical roots in Japan through centuries of experience in producing fine quality kamaboko type products. Surimi is a Japanese term denoting ground fish meat paste. It is stabilized myofibrillar proteins obtained from mechanically deboned fish flesh that is washed with water and blended with cryoprotectants. Large scale production of surimi became a commercial reality since Nishiya *et al.* (1960) at Hokkaido Fisheries Research Station of Japan, discovered the role of cryoprotectants in preventing freeze denaturation of proteins in Alaska pollock (*Theragra chalcogramma*) muscle. This development together with the introduction of a new generation of surimi based shellfish analogue products in the West led to world wide recognition of this technology.

The surimi industry has changed dramatically over the past decades. A decrease in Alaska pollock harvests, from over 6.5 million metric tons in the late 1980's to less than 3 million metric tons since the year 2000, has opened the door for utilization of new species in the surimi industry. Several advances have been made in surimi production, thereby greatly improving the economics of the production process.

The surimi supply fluctuated between 4, 50, 000 and 5, 50, 000 metric tons during the 1990's, but increased slightly in recent years. According to FAO statistics published in 2004, global production of surimi was between 8, 60, 000 and 11, 50, 000 metric tons. The five main countries producing surimi are Japan, South Korea, Thailand, USA and China. The European Union has now emerged as a major producer. In the year 2002 it was estimated that about 2, 00, 000 tons of surimi was manufactured from tropical fish of which India's contribution was about 20 %, which may work out to roughly 40, 000 tons.

It is necessary to distinguish surimi from minced fish. When fish flesh is separated from bones and skin and comminuted, it is called minced fish, the starting material for surimi production and an ingredient for some processed fish products such as fish sticks and cakes. Although whole fish can be preserved long by freezing, minced fish meat has a very low shelf life. The proteins exposed during picking and mincing get easily denatured resulting in excessive drip and a fibrous structure within a short period, making the mince unsuitable for preparing various products.

When the minced fish is water washed to remove fat and water soluble components it becomes raw surimi. This material possesses enhanced gel forming, water holding, fat binding and other functional properties relative to minced fish. However, the myofibrillar proteins in the raw surimi will lose their functional properties rapidly once they are frozen as in the case of ordinary minced meat. When the raw surimi is mixed with cryoprotectants such as sugars or sugar alcohols and quick frozen into a block form, frozen surimi is obtained. The myofibrillar proteins in the frozen surimi will retain their functional properties for several months if properly stored.

Washing is an essential step in removing water soluble proteins, primarily sarcoplasmic proteins, which is thought to impede the gel- forming ability of surimi, fat and other impurities that reduce product quality. Myofibrillar protein, the primary component that possesses the ability to form a three- dimensional network, constitute approximately 65 % of the total proteins in minced fish meat. Sarcoplasmic proteins exist in fluids within and between muscle fibers and constitute 20 to 30%. This includes many metabolic enzymes that diminish the stability of functional proteins during storage. They are soluble in water and in salt solution of low ionic strength. Being highly water soluble, they influence neither the water holding capacity nor the texture of fish muscle (Susuki, 1981). Upon heating sarcoplasmic proteins coagulate and adhere to myofibrillar proteins. This coating will adversely affect gel strength. Stroma proteins, viz., collagen and

elastin form the connective tissue and are not soluble in water, acid, alkali, or neutral salt solution. They do not possess the functional properties required for various products. On heating collagen get gelatinized, and affect gel strength. The presence of high lipid content has certain negative implications in the storage, processing and stability of fish muscle. So all these components are to be removed from fish mince by washing.

The number of washing cycles and the volume of water used vary with fish species, the freshness of fish, structure of washing unit, and the desired quality of surimi. Lin *et al.* (1995) reported that for a shore-side operation, 29.1 liters (L) of waste water was generated to produce 1 kilogram (kg) of surimi. An effective washing process can now be accomplished with two washing cycles with water-meat ratios of less than 2:1. In comparison, at-sea processors can achieve the same washing effect with less water than shore based processors due to the difference in the freshness of fish (Park, 2000).

For commercial surimi production, fish mince is repeatedly washed with chilled water and dewatered to produce high-quality surimi. The water mince ratio and the number of washing cycles can differ, depending on the desired surimi quality. Soluble components are in the waste water in varying concentrations, depending on the wash ratios and the processing steps. Wu *et al.*, 1991, and Lin and Park, 1996 reported that myofibrillar proteins are also readily solubilized under certain conditions and lost to waste water during processing. As the washing process progresses, the myofibrillar proteins are more likely to be lost in the waste streams. In a typical surimi operation, only 50 to 60% of the myofibrillar proteins are often retained through washing and dewatering process. Approximately 40 to 50% myofibrillar proteins can be lost in soluble or insoluble forms due to factors such as changes in pH and ionic strength, proteolysis and mechanical forces in mincing, washing, screening and screw pressing.

Surimi wash water (SWW) presents a different set of problems of waste disposal because of their high volumes and low concentrations of solid material.

Surimi waste water contains about 0.5 to 2.3% total proteins composed mainly of sarcoplasmic proteins with small amounts of myofibrillar proteins, myosin and actin (Lin and Park, 1996, Park and Morrissey, 2000, Morrissey *et al.*, 2000, and Savant and Torres, 2003). Because surimi waste water is high in organic loads direct discharge can cause potential negative environmental impacts, thus threatening aquatic organisms in the water. Water shortages, stringent environmental regulations, and the rising cost of water disposal have caused concern among surimi manufacturers. These increasing concerns have lead to research in protein recovery from surimi waste water. This not only produces protein for food and feed, but also generates treated water for potential reuse in seafood processing plants; reduces.negative environmental impact and lowers the cost of waste disposal.

Coagulation of proteins by different techniques and subsequent separation by centrifugation is the common technique used for solid recovery. Several methods for solid recovery have been practiced, viz., pH modification using acid or alkali, heat coagulation, ultra filtration, micro filtration, flocculation using synthetic poly electrolytes, chitosan-alginate complex, etc. As the pH approaches the isoelectric point (4.2 to 5.0), due to the lack of electrostatic repulsion between the protein particles, they tend to aggregate, grow in size, and finally precipitate. The precipitated proteins can then be separated from waste water. Heat causes unfolding of protein chains and they precipitate, which can then be separated.

The present study was aimed at testing the efficiency of pH reduction and heat coagulation, in the recovery of solids from surimi waste water obtained during water leaching of meat of tilapia (*Oreochromis mossambicus*). The study also included assessment of the quality of water after recovery, the quantity of solids recovered as well as the preparation of a fish feed with the recovered solids. Objectives such as recovery of proteins from surimi waste water, reducing environmental pollution, and generating protein that can be potentially used as an animal feed component have been targeted.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Proteins found in the fish muscle have been classified into different categories according to their distribution, organization, solubility and function in the living muscle tissue. Based on solubility the fish proteins are classified into three categories viz., sarcoplasmic proteins, myofibrillar proteins and stroma proteins (Goll *et al.*, 1977).

Striated fish muscle is composed of muscle fibers, which in turn contain myofibrils. The myofibrils are constructed of end-on-end contractile units called sarcomeres, which contain three types of filaments – thick, thin and connecting – arranged in such a fashion as to impart the striated appearance of muscle under the microscope. Suzuki (1981) reported that the predominant protein in the sarcomere, found in the thick filament system, is myosin, constituting approximately 55 to 60% of total myofibrillar protein content. The A bands of the myofibrils are composed of thick filaments and the I bands composed of thin filaments as shown in Fig.1. Each thick filament is formed from an ordered arrangement of about 400 myosin molecules.

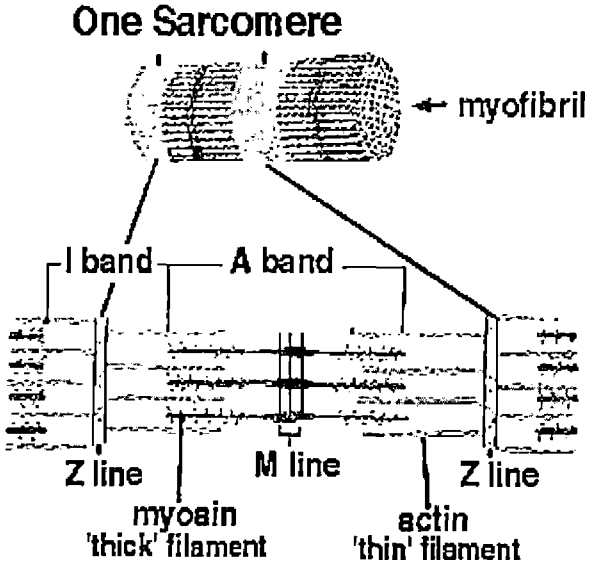

Fig. 1: Structure of myofibril

Surimi is concentrated myofibrillar protein obtained from fish flesh after several washings with cold water. In industrial surimi manufacturing process, minced flesh is repeatedly washed with chilled water to remove sarcoplasmic proteins and impurities such as lipids to produce a stable product for frozen storage. Surimi is prepared by water leaching of mechanically separated fish muscle followed by the addition of cryoprotectants to improve protein stability during frozen storage (Lee, 1984).

Surimi processing, which includes several unit operations, can be divided into two major stages according to Fig.2 (Morrissey *et al.*, 2000). The first stage includes heading, gutting, deboning and mincing, which prepare the fish mince for washing and refining operations of the second stage. Streams of water are injected into the stage to remove fish fluid and the muscle/ meat that adheres to the machines, for transporting skins, backbones and viscera from filleting and deboning machines to a scrap delivering system. The overall fresh water consumption can be largely reduced by using recycled water for transporting scraps. In the second stage, fish mince is repeatedly washed with chilled water and dewatered to produce high quality surimi. Here water is used for removing water soluble proteins, fats, pigments etc. that are not desirable for the final quality of surimi. The mince: water ratio (w/v) and the number of washing cycles can differ, depending on the desired surimi quality.

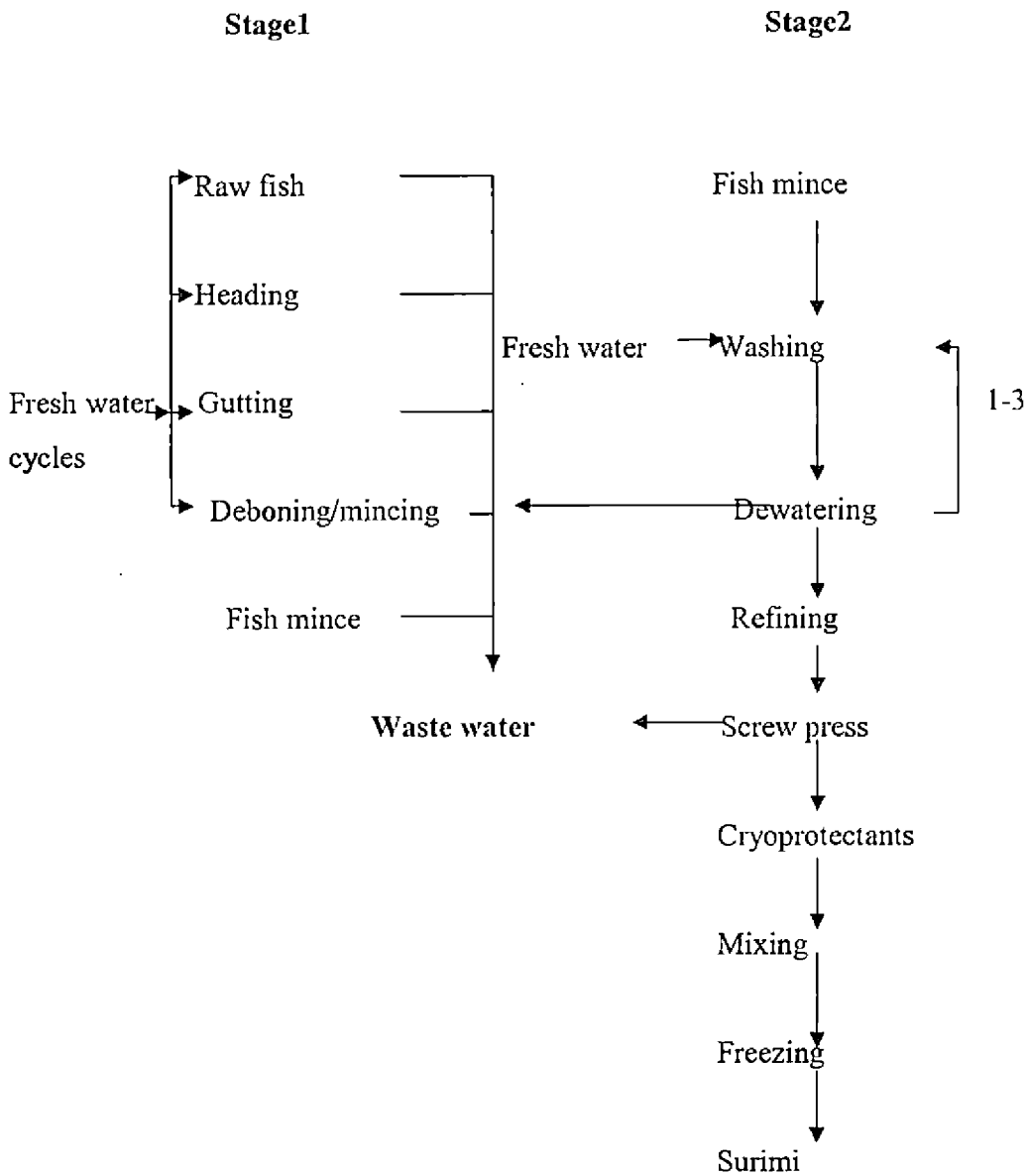

Fig. 2: Flow chart showing generation of surimi processing waste water

(Morrissey *et al.*, 2000)

2.1 Washing and dewatering

Washing is a critical step in producing high quality surimi. Lanier (1986) reported that the washing procedure is of great importance on the final quality of surimi, not only for removing fat and other undesirable material such as blood, pigments, and odoriferous substances, but more importantly for increasing the concentration of myofibrillar proteins (actomyosin) thereby improving gel-forming ability and decreasing protein denaturation during frozen storage.

A proper washing process, therefore, is vital to achieve high quality surimi with high recovery. An insufficient washing process could result in a substantial loss of gel quality during frozen storage. Gomez and Mendes (1997) conducted studies to determine the effects of wash water parameters such as pH, hardness and sodium tripolyphosphate (STPP) content on water holding capacity and gelation properties of sardine (*Sardina pilchardus*) mince. Two experiments designed by response surface methodology were carried out. Water holding capacity of the mince was maximum when washed in the pH range of 6.5 to 7.0; with STPP greater than 1.7 g/L and $CaCO_3$ between 30 and 60 mg/L. Variation of water hardness did not induce any significant changes in rheological parameters. Use of relatively high concentrations of STPP in the leaching water contributed to a detrimental effect on breaking force and consequently also on gel strength.

A beneficial decoloration effect resulted for horse mackerel mince washed with ozonized water within 10 to 20 min, but a longer washing time was required to improve the colour properties when cold water or alkaline solution was used (Chen *et al.,* 1997). Increase in pH as well as improvements in gel forming ability occurred for mince washed with alkaline solution. Maximal gel strength was obtained for surimi washed for 90 min. A marked decrease in pH and an undesirable gel strength of mince as well as oxidation of the fish oil occurred during ozone treatment. Since the salt-soluble protein concentration increased for

all minces washed with the three methods, improvement in gel-forming ability of washed mince was attributed to increase in pH rather than to oil removal. According to Mendes *et al.* (1998) leaching horse mackerel meat using a solution of tetrasodium pyrophosphate (0 to 0.2 %) and $CaCO_3$ (50 mg/L) at a pH of 6.5 resulted in good gelatin characterized product.

Effects of washing and storage on the quality of *Selaroides leptolepis* and *Aristichthys nobilis* surimi kept at -20 °C for 24 weeks were investigated by Siah *et al.* (1998). Results showed that twice washed surimi of both species were generally more stable than once washed and unwashed surimi. The quality in terms of texture, colour, elasticity, moisture content, pH and salt soluble protein value for all samples showed significant decrease during storage. There were increases in expressible moisture, trimethylamine, total volatile base nitrogen and thiobarbituric acid values.

Studies conducted by Sankar and Ramachandran (2002) on mrigal flesh indicated absorption of water by 1 to 3 %, loss of fat by 49 % and loss of salt soluble proteins by 35 % upon washing. Approximately 75 % of salt soluble proteins were retained after three washing cycles. Bindu *et al.* (2004) prepared surimi from black tilapia (*Oreochromis mossambicus*) after storing the fish in ice for different periods. Two washing cycles were given under two different mince-water wash ratios, viz., 1:2 and 1:3. Two successive washings at a mince: water ratio of 1:2 (w/v) for a period of five minute each was found to be ideal for optimum yield and quality of the surimi.

The effect of the number of water washing cycles on the proximate composition as well as physicochemical and functional properties of proteins from threadfin bream meat was studied by Karthikeyan *et al.* (2006). There was a significant decrease ($P < 0.05$) in the contents of proteins (3.03% to 2.06%), fat (3.45% to 1.23%), ash (1.13% to 0.15%) and non-protein nitrogen (315.97 mg/100g to 83.33 mg/100g) with the number of washing cycles.

Washing efficiency is often affected by various factors. In addition to mince: water ratio and age of fish, there is the shape of washing tank (round vs. square), the speed of the agitator, the shape of the agitator (vertical vs. horizontal) and water temperature. Square shaped tanks seem to work better than round shaped tanks because the former can generate a counter current washing effect. When the agitating paddle is placed horizontally, rather than vertically, the washing efficiency is higher. When the agitator is operated too fast, it might also result in a temperature rise as well as difficulty in dewatering by screw press. The optimum speed for agitation must be determined based on specific operations (Park, 2005).

2.2 Recovery of solids from surimi wash water

During washing usually at least 3 times water (W/V) is used in 3 cycles, releasing huge amounts of water into our water bodies which can result in massive eutrophication. Increasing concern over the negative impact of direct waste water discharge has led to research in protein recovery from SWW. This not only produces proteins for food and feed but also generates treated water for potential reuse in seafood processing plants. Recycling of surimi wash water is gaining importance due to rising utility cost, limited water resources, and pollution problems associated with disposal. Considerable research has been reported concerning water reuse and conservation.

Ultrafiltration is the system of choice when it comes to producing protein concentrates and isolates with good functional properties (Horton *et al.*, 1972). The problem early on with ultrafiltration involved severe fouling of membranes (Morr, 1976). Jaouen and Quemenuer (1992) noted that processing of surimi wash water by ultrafiltration, with out pretreatment was not practical. Attempts to combine ohmic heat treatment with ultrafiltration on surimi wash water was also investigated. Although several flocculation agents such as ferric chloride and

aluminium sulphate were used in protein precipitation, these flocculants were discarded due to their high toxicity to humans even at low concentrations (Marti *et al.*, 1994).

Lin *et al.* (1995) conducted a study on protein recovery from pacific whiting (*Merluccius productus*) surimi wash water using ultra filtration and micro filtration. Wash water was collected separately for all washes including press water. He observed that waste water in successive discharge points revealed decreasing protein, non protein nitrogen, fat, and ash. Results from SDS-PAGE showed that a considerable amount of myofibrillar proteins, actin and myosin was lost in the waste water during washing and dewatering. Surimi with 10% replacement of recovered protein had the same gel quality as regular surimi with respect to gel hardness, elasticity, water retention, and colour. The gel made with recovered protein had better color and quality than regular surimi gels. The surimi production rate could be increased by 1.7% by adding the recovered protein back to the production line without diminishing the surimi functionality. The aerobic plate count (APC), chemical oxygen demand (COD), turbidity and protease levels of waste waters were substantially reduced by ultra filtration. However, proteins concentrated by ultra filtration had considerable dark color and strong odours.

Benjakul *et al.* (1997) recovered proteinase from Pacific whiting surimi wash water by ohmic heating, ultrafiltration and freeze drying with overall yield of 0.83g/L of surimi wash water and 78% recovery of activity. Different applied voltages (50, 70 & 90 V) showed no differences in efficiency for removing protein and retaining cathepsin L activity. Cathepsin L activity reached its maximum after ohmic heating to 55^0C whereas cathepsin B activity decreased constantly with increased temperature. They observed a constant decrease in protein content with increase in temperature from 45^0C to 60^0C and holding time up to 10 minutes. The highest retention of both total and specific activity of Cathepsin L was obtained with the treatment at 55^0C for 3 minutes. Under these conditions they could recover 193% activity from surimi wash water although a

large amount of activity was lost by the subsequent steps of ultrafiltration and freeze drying.

Zhang *et al.* (1999) worked on the recovery and utilization of water soluble fish proteins from surimi washings from silvercarp mince using sodium alginate. The recovered protein was effectively utilized by mixing with surimi in an appropriate proportion. He observed that efficiency of protein recovery and percentage of COD depression were 80% and 50% respectively at an optimum pH of 4.3 +\- 0.6 and the quantity of sodium alginate added depended on the content of protein in the washings. He also reported that the gel strength of fish sausage prepared from surimi with which 5% of the recovered protein was mixed up was the same as that from surimi unmixed.

Kaur *et al.* (2000) concentrated anserine and carnosine in surimi wash water collected from surimi plants, by ultra filtration. Tests showed that anserine and carnosine were present in high concentration (>200mg/L) in the first two stages of surimi processing. Treatment of the samples by ultra filtration removed the majority of large protein particles in the wash water, but caused some reduction in the dipeptide concentration.

Dewitt and Morrissey (2002) conducted experiments on recovery of proteases from Pacific whiting (*Merluccius productus*) surimi wash water. They observed maximum protease activity and acidification at pH 6 using either HCl or 0.1% L-ascorbic acid followed by heat treatment at 60^0C. They also reported that acidification to a pH of approximately 4.5 with either HCl or L-Ascorbic acid along with heating maximized protein removal from wash water and the type of acid applied did not significantly affect final protein content of unheated wash water. They showed that large molecular weight proteins that interfered with ultra filtration could be removed without decreasing protease activity by first adjusting wash water acidity to pH 6 and then applying a rapid heat treatment at 60^0C.

Kim *et al.* (2003) observed that Pacific Whiting protein solubility was significantly affected as the pH shifted away from isoelectric point (pH 5.5). Chen *et al.* (2003) studied the feasibility of using native isoelectric focusing electrophoresis (IEF) of water- soluble sarcoplasmic proteins in identifying puffer fish species. The results showed that water soluble puffer fish muscle proteins fell in the region with isoelectric pH values of 3.5 to 5.2.

Raweewan *et al.* (2004) recovered proteins from surimi wash water collected from processing plants by ultra filtration and micro filtration, and then partially purified by crystallization. SDS-PAGE study showed that the range of molecular weight of soluble proteins in surimi wash water was 10-100kDa. Choi and Kim (2005) also observed that solubility of meat protein of Jack mackerel was significantly affected by pH shifting from isoelectric point.

Wibowo *et al.* (2005) used chitosan – alginate (Chi-Alg) complex for protein recovery from surimi wash water. Prior to flocculation, surimi wash water was centrifuged for 20 minutes at 3100Xg and 4^0C to remove insoluble proteins which was freeze dried to obtain protein rich diet (p1). Supernatant was then adjusted to pH 6 using 1M HCl. The soluble protein remaining in the pH adjusted supernatant was recovered using Chi-Alg complex.The solids were recovered using 20 minutes centrifugation at 3100 Xg and 4^0C, freeze dried and stored at -39^0C to obtain a second protein-rich pellet (p2). Protein content of p1 and p2 was 61.4% and 73.1% respectively on dry weight basis. Histidine, lysine, methionine, and phenyl alanine in the recovered proteins were higher than in commercial feed ingredients. In a rat feeding trial, a casein control and diets formulated with surimi wash water protein at 10% and 15% substitution showed no significant difference in protein efficiency ratio. Another work done by Wibowo (2005) analyzed the effect of Chi-Alg complex concentration and treatment time on protein adsorption. Chitosan effectiveness was increased by complexing with alginate and by adjusting complex concentration and treatment time. Flocculation at 20^0C with Chi-Alg at a 0.2 mixing ratio added as 20, 40, 100 and 150 mg/L surimi

wash water was aided by 5 minutes agitation at 130 rpm and then held at the same temperature for 30 minutes, 1h, and 24 h. Turbidity measurements, protein determination and qualitative FTIR analysis confirmed protein adsorption in surimi wash water.

Velazquez *et al.* (2007) evaluated the effect of adding insoluble proteins recovered from Pacific whiting (*Merluccius productus)* surimi wash water using Chitosan-Alginate complex on mechanical and functional properties of Allaska pollock surimi gels. Results obtained showed that texture profile analysis, and puncture test increased significantly by increasing the amount of insoluble proteins added. Works done by Ramirez *et al.* (2007) showed that incorporation of insoluble solids from Pacific whiting (*Merluccius productus)* surimi wash water to commercial surimi increased the water holding capacity of the paste prepared with 2% salt added.

Huang *et al.* (2007) developed a batch type ohmic heating device to investigate the possibility of coagulating fish protein from frozen fish mince wash water. At constant voltage (90 VAC), the temperature of wash water samples was raised to different set points (40, 50, 60, 70 and 80 ^{0}C, respectively). Effect of heating on coagulation of proteins and removal of COD, TS and TSS was investigated. When the temperature reached 70^{0}C, 33.0%, 59.3%, 33.3% and 92.1% protein, COD, TS and TSS, respectively, were removed from the wash water. Holding samples at constant temperatures for longer time periods did not improve solids removal, except at 40^{0}C. The highest heating temperature for effective coagulation of proteins and removal of solids is 70^{0}C. The relationship between heating temperature and heating time followed a second order polynomial model. Apparent electrical conductivity and energy consumption increased linearly with the heating temperature. At the early stage of heating, almost all electric energy was converted to heat energy. As the temperatures rose, energy efficiency began to decrease linearly with the temperature. Overall energy efficiency was above 86%. He reported that the wash water soluble proteins could be effectively removed; however, the proteolysis due to heat could not be neglected.

Kajanapongkul *et al.* (2008) developed a batch ohmic heater to investigate the reduction of solids contained in thread fin bream surimi wash water as well as to examine the electrical conductivity of the samples. It was found that the optimum heating temperature was 70^0C. After heating to 50, 60 and 70^0C, the remaining proteins in the samples were reduced to 63%, 49% and 42%, respectively compared with the initial values. Further heating to 80^0C resulted in just a slight reduction of proteins (39%). At 70^0C, the biological oxygen demand (BOD) value of the sample was reduced to 23%. These results suggested the potential of using ohmic heating to improve surimi wash water quality. A continuous ohmic heating system was developed by Kanjanapongkul *et al.* (2009) to coagulate proteins from surimi wash water and to reduce BOD of the waste water. Samples were treated under different conditions (electric field strength of 20, 25 and 30 v/cm; flow rates of 100, 200 and 300 cc/min). After heating, the samples were centrifuged and remaining protein in the supernatant was measured. This technique could coagulate 60% proteins from surimi wash water. Bourtoom *et al.* (2009) obtained maximum precipitation at pH 3.5 for *Nemipterus hexodon* surimi wash water; close to isoelectric point of this type of proteins.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Standardization of procedures

Fresh tilapia (*Oreochromis mossambicus*) was collected from Puthuveypu immediately after capture and was brought to the laboratory within 6 hours chilled in ice. Meat was picked and washed for collection of surimi wash water, hereafter to be referred as SWW. The optimum pH and optimum temperature for maximum coagulation was standardized according to the following procedure (Fig. 3).

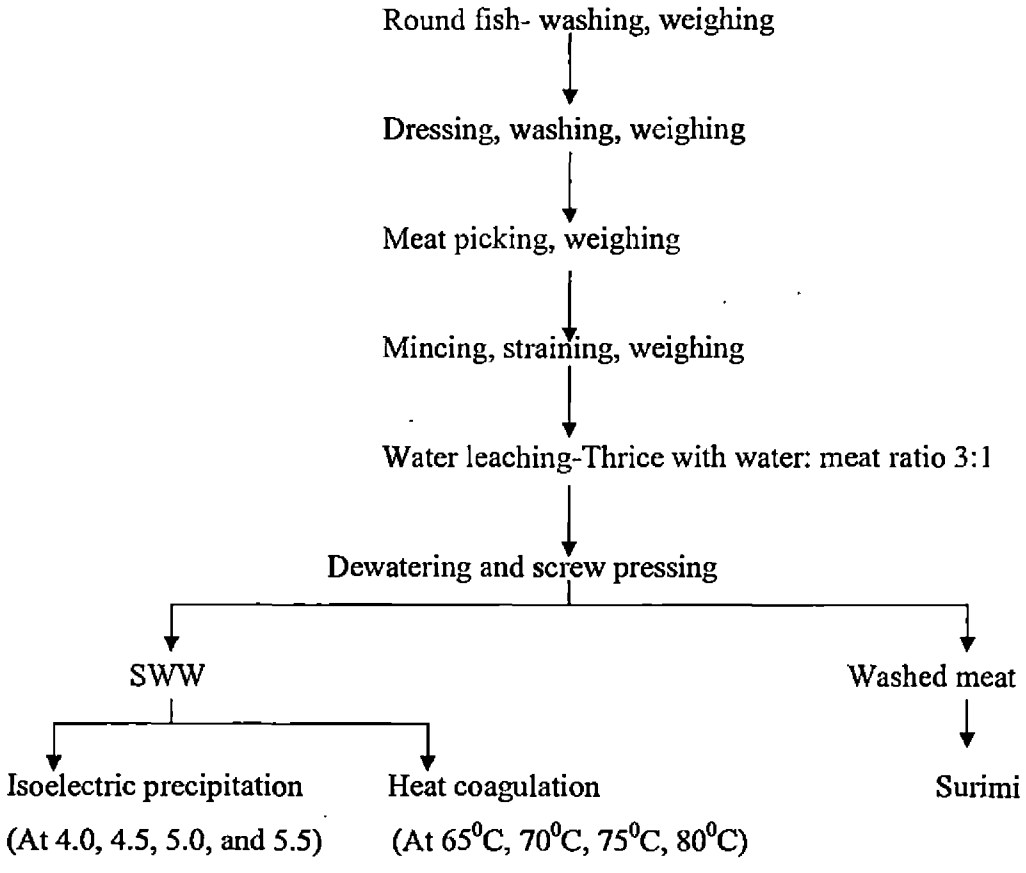

Fig. 3: Flow chart for standardization of pH and temperature for recovery of solids from SWW

The fish was headed and gutted followed by thorough washing in iced potable water. The fish was then filleted and the meat manually picked. The picked meat was minced in a meat mincer using a disc of 0.55 cm diameter perforations. The meat was strained using an extruder with perforations of 0.30 cm diameter. The meat was then water leached using iced water, added at the rate of three times the weight of meat and at a temperature of about 15 °C, and stirred for a period of 10 min using a mechanical stirrer. The meat particles in the suspension were settled and the supernatant was decanted and wash water was collected. Leaching was repeated two more times. For the last wash a solution of 0.05 % NaCl was used along with water. The material was dewatered by wrapping in a cloth bag and pressing using a basket-type press to a final moisture content of about 80 %. The press water was also collected and all the water samples were mixed together. These water samples termed surimi wash water (SWW), was divided into 40 lots of 50 ml each. Each 5 lots were subjected to different pH (4.0, 4.5, 5.0, 5.5) using 10% HCl and temperature (65^0C, 70^0C, 75^0C, 80^0C). After protein precipitation, these lots were separately centrifuged in a refrigerated centrifuge for 20 minutes at 3100 Xg and 4^0C to recover solids (Lin *et al.*, 1995). The precipitate after recovery was allowed to dry in a tray drier at 60 to 65^0C to a moisture content of 80%. The recovered solids were then weighed to determine the maximum recovery. Both optimum pH and temperature were thus established on the basis of the weight of recovered solids keeping all conditions common.

3.2. Recovery of solids from SWW

As for standardization, meat was picked from fresh tilapia and washed for collection of SWW. SWW was then subjected to optimized pH and temperature for protein coagulation according to the following flow chart (Fig.4)

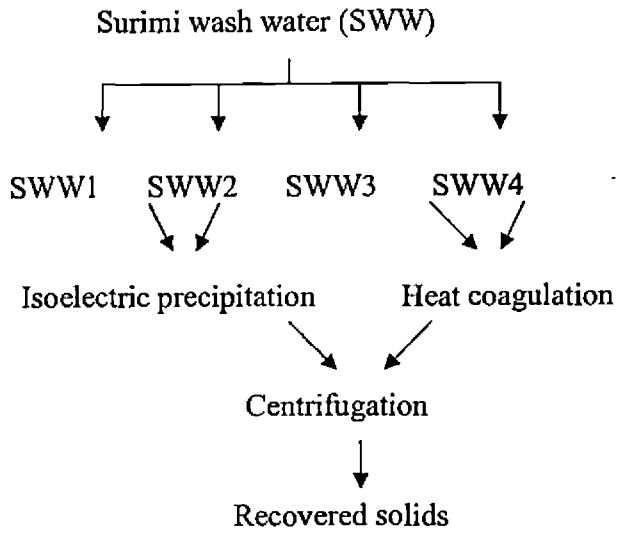

Fig. 4: Flow chart for recovery of solids from SWW

SWW was collected as per above procedure and divided into 4 lots. 2 lots were subjected to optimum pH for isoelectric precipitation and another 2 lots were subjected to optimum temperature for heat coagulation. After protein precipitation, these lots were separately centrifuged in a refrigerated centrifuge for 20 minutes at 3100Xg and 4^0C to recover solids (Lin *et al.*, 1995). The precipitate after recovery was allowed to dry in a tray drier at 60-65^0C to a moisture content of 80%. The recovered solids were then weighed to determine the percentage recovery for pH and temperature. The whole process was done in quadruple to check statistical significance.

3.3. Compositional profile of fish mince and recovered solids

Compositional profile of fish mince before and after water leaching was determined to check the effect of washing on composition of fish mince. Compositional profile of recovered solids by both treatments were also analyzed to compare the compositions of both.

3.4 Water analysis

Quality of water before and after recovery were analysed by conducting various tests such as proximate composition, chemical oxygen demand (COD), biological oxygen demand (BOD) and total plate count (TPC) of SWW before and after recovery. Efficiency of various treatments in reducing proximate composition, BOD, COD and TPC were also analyzed.

3.5 Preparation of fish feed

Fish feed was formulated with the ingredients given in Table 1.Three types of feeds were formulated viz., one with recovered solids by heat coagulation , another with recovered solids by isoelectric precipitation and the third, a control feed formulated with clam meat as animal protein source. The ingredients were mixed thoroughly with water to form a dough. The dough was cooked for 20 minutes at atmospheric pressure, pelletized using a hand pelletizer and was dried to a moisture content of 10%.Proximate composition of the test feeds were compared with the control feed.

Table 1: Ingredients for preparation of fish feed

Ingredients	Percentage required
Animal protein	25%
Ground nut oil cake	25%
Rice bran	40%
Tapioca flour	10%
Water	1.25 times

3. 6 Tests

Various tests were conducted to determine proximate composition, BOD, COD and TPC. Table 2 gives the list of tests conducted.

Table 2: List of tests conducted for the study

Tests	Samples for testing
Proximate composition (Moisture, protein, fat and ash)	Minced meat, washed meat, SWW, recovered solids, water after recovery and formulated feed
BOD	SWW and water after recovery
COD	SWW and water after recovery
TPC	Minced meat, washed meat, SWW, recovered solids and water after recovery

3.6.1 Moisture content

The moisture content was determined by the AOAC (1975) oven drying method. A sample of about 2g was weighed in a pre-weighed tared petridish. The dish was placed in a hot air oven at a temperature of 100°C for a period of six hrs, cooled in a dessicator and weighed. Drying was continued until a constant weight was obtained and the moisture content was calculated as percentage loss of weight.

3.6.2 Ash content

The method of AOAC (1984) was followed for ash content estimation. About 0.5g of the sample was transferred to a pre-weighed silica crucible. The sample was carbonized by burning at low red heat and was placed in a muffle furnace at a temperature of 550°C for about four hrs until a white ash was obtained. Crucibles were weighed after cooling in a dessicator. The weight of ash was expressed as a percentage of the initial sample weight. For water samples 50ml water was used for carbonization.

3.6.3 Crude fat content

The method of Radin (1981) was followed for determining crude fat content. About 1g of sample was taken in a mortar and homogenised with 18 ml of the extraction solvent (hexane: isopropanol :: 3:2, v/v). It was then filtered into a pre-weighed beaker. The residue was washed two or three times with minimum volume of solvent mixture. The solvent was evaporated off on boiling water bath, then cooled to room temperature in a desiccator, and weighed. From the difference in weight the percentage lipid content was calculated.

For water samples petroleum ether was used as extraction solvent. About 5ml of the sample was thoroughly mixed with 10ml petroleum ether in a preweighed test tube. The solvent was evaporated off on boiling water bath, then cooled to room temperature in a desiccator, and weighed. From the difference in weight the percentage lipid content was calculated as before.

Soxhelet apparatus was used to determine crude fat in feed samples. About 2g of dried feed was taken in an extraction thimble and the thimble was placed inside the apparatus with required quantity of petroleum ether. The apparatus was connected to dry flask of solvent and to a condenser. Electric heating was adjusted so that the solvent siphons 5 to 6 times/h. Extraction was continued for 4h. After

extraction the system was brought to room temperature. The liquid extract was them transferred to a pre weighed dish and the solvent was evaporated off. The dish was then cooled in a desiccator and weighed. From the difference in weight, the percentage lipid content was calculated.

3.6.4 Total nitrogen and crude protein contents

Total nitrogen and crude protein contents were estimated by the Micro Kjeldahl method (AOAC, 1984). About 1g of the well-minced wet sample was transferred to a kjeldahl flask of 100 ml capacity. A few glass beads and a pinch of digestion mixture (K_2SO_4 and $CuSO_4$ = 8:1) and 10 ml concentrated sulphuric acid were also added. It was digested over a digestion unit (Pelican make) until the solution turned colourless. To the digested and cooled solution distilled water was added in small quantities with intermittent shaking and cooling until the addition of water did not generate heat. It was transferred quantitatively to a 100 ml standard flask and made upto the volume. Two ml of the solution was pipetted out into the reaction chamber of the micro kjeldahl distillation apparatus. Ten ml of 40% (w/v) sodium hydroxide solution was added to the sample. Distillation was carried out for a period of two minutes and ammonia liberated was absorbed into 2% boric acid solution taken in a conical flask containing 1-2 drops of Tachiro's indicator. The amount of ammonia liberated was determined by titrating with $N/_{70}$ standard sulphuric acid. Total nitrogen and crude protein contents were calculated as follows.

$$\text{Total nitrogen (\%)} = \frac{V \times 14 \times 100 \times 100}{1000 \times 70 \times 2 \times W}$$

where, V = volume of standard sulphuric acid required for titration in ml,
and W = weight of sample taken in g or volume of sample taken in ml.

Crude protein (%) = % total nitrogen x 6.25

3.6.5 Biological oxygen demand (BOD)

Samples were diluted by pipetting 10ml of water samples into 500ml distilled water. From this stock samples were taken for estimation of initial oxygen level and final oxygen level after incubation. Incubation was carried out at 20^0C for 5 days. Dissolved oxygen level was determined using Wringler's reagent. Water samples were slowly siphoned out into different BOD bottles without entrapping air and were allowed to overflow the bottle. Two bottles were taken for each sample of which one was incubated for 5 days.

For determining dissolved oxygen (DO), 1ml of manganous solution (40g manganous chloride tetrahydrate) was added first to water samples. One ml of alkaline iodide was also added. The bottle was carefully stoppered and tilted upside down. One ml of 50% sulphuric acid was slowly added to the bottle and the bottle was again stoppered and tilted sidewise. From these reagents fixed BOD bottles 50 ml of sample was taken and titrated against 0.01 N sodium thiosulphate. DO of potable water was also calculated in the similar manner.

DO and BOD were calculated using the following formulae

$$\text{DO (mg/BOD bottle)} = \frac{8 \times 0.01 \times \text{Titrevalue} \times \text{Volume of BOD bottle}}{50}$$

$$\text{BOD (mg/L)} = \frac{\text{Initial DO of bottled sample} - \text{Final DO of incubated sample}}{\text{Volume of sample in BOD bottle}}$$

3.6.6 Chemical oxygen demand (COD)

Water samples were suitably diluted. For wash water and water after recovery by pH shift 25ml was made up to 100ml using distilled water. For diluting water after recovery by heat treatment 50ml was made up to 100ml using distilled water. This made up solution was taken in a conical flask and 5ml of 5% sodium hydroxide solution and 20ml of 0.01N potassium permanganate were added to it. The contents of the flask were mixed thoroughly and heated in a water bath for 20 minutes and cooled under running water. To this mixture 5ml of 25%sulphuric acid and 10ml of 0.1M potassium iodide were added. This was then titrated against 0.02N sodium thiosulphate solution using starch as indicator. COD was calculated using the formulae

COD (mg/L) = 0.8 x N x 100 (b-s)

where N=Normality of sodium thiosulphate

b= ml of sodium thiosulphate solution used for titrating the blank

s= ml of sodium thiosulphate solution used for titrating the sample

3.6.7 Total plate count (TPC)

All media and diluents were sterilized by autoclaving at a temperature of 121°C for 15 min and all glass wares at 160°C in hot air oven for 2h. Total plate count was determined according to the method of Surendran *et al.* (2006). A sample of 1g was aseptically transferred to a sterile blender and homogenized with 90 ml sterile diluent (normal saline). Appropriate serial decimal dilutions of the homogenate were made using the diluent and dilutions of 10^{-1}, 10^{-2} and 10^{-3} were plated by pour plate technique in triplicate. For water samples 1ml of sample was aseptically transferred to 9ml sterile diluent and serial dilution was made and plated as before. The medium used was Nutrient Agar of SRL make (composition in g/l: beef extract-10, peptone-10, NaCl-5 and agar-12). The plates after solidification were inverted and incubated at a temperature of 37°C for 24 h.

Plates showing 30 to 300 colonies were used for counting the colonies. TPC was calculated using the formula:

$$TPC = \frac{\text{Average number of colonies x dilution factor}}{\text{Weight or volume of the sample taken}}$$

The count was expressed as number of colony forming units (cfu) / g sample or cfu/ml sample.

3. 7 Statistical Analysis

The data were statistically analyzed using descriptive statistics, student's t – test and ANOVA. The analysis was carried out using SPSS (ver. 15. 0).

RESULTS

4. RESULTS

4. 1. Standardization of procedures

The temperature for heat coagulation and pH for isoelectric precipitation were standardized as discussed under section 3.1. Quantity of recovered solids from 50 ml SWW by heat coagulation and pH shift are given in Tables 3 & 4, respectively. Optimum temperature and pH were selected based on maximum recovery. The trend in the recovery of solids by the two methods are shown in Fig. 5 & 6, respectively. The results of statistical analysis using ANOVA are given in Table 5 & 6 for heat coagulation and for isoelectric precipitation, respectively and pair wise comparison using student's t test are given in Table 7 & 8, respectively.

A temperature of 75^0C yielded 2.13 g of recovered solids from 50ml SWW which was found to be the maximum recovery. At 65^0C the quantity of solids recovered was as low as 0.88g. Hence a temperature of 75^0C appeared to be optimum particularly since a higher temperature of 80^0C did not yield higher recovery levels. In the recovery of solids from SWW by isoelectric precipitation it was seen that pH at 5.0 yielded 0.70g of recovered solids which was found to be the maximum recovery. Hence pH 5.0 appeared to be an optimum pH for isoelectric precipitation. Higher pH levels of 5.5 and lower pH values of 4.5 yielded less than 50% of what was recovered at pH 5.0.

Analysis of variance was carried out for recoveries by heat coagulation and recovery by isoelectric precipitation. The results of ANOVA for both standardizations showed that there is significant difference between the solid recovery at varying levels of temperature and pH. Pairwise comparison of average quantity of recovered solids by heat coagulation using different temperatures and by isoelectric precipitation using different pH were done, using student's t test. The results showed that quantity of recovered solids at 75^0C was significantly

higher ($p<0.05$) than that at other temperatures (Tables 3 & 7). Similarly pH of 5.0 yielded significantly higher ($p<0.05$) solids than at other levels of pH (Tables 4 & 8). It was also observed that all temperature pairs except (70^0C and 80^0C) showed statistical significance ($p<0.05$). Similarly in the case of pH statistical significance was obtained at all pH pairs except (4.5 and 5.5). Hence 75^0C was selected as the optimum temperature for heat coagulation and a pH of 5.0 was selected as optimum pH for isoelectric precipitation.

Table 3

Recovery of solids from SWW by heat coagulation.

Temperature (^{0}C)	No. of observations	Recovered solids (g)	
		Mean	SD
65	5	0.88	0.14
70	5	1.68	0.27
75	5	2.13	0.56
80	5	1.51	0.19

SD -Standard deviation

Table 4

Recovery of solids from SWW by isoelectric precipitation

pH	No. of observations	Recovered solids (g)	
		Mean	SD
4.0	5	0.06	0.02
4.5	5	0.33	0.12
5.0	5	0.70	0.17
5.5	5	0.28	0.12

Table 5

ANOVA for standardization of temperature

	Sum of squares	df	Mean square	F	Probability (p)
Between groups	4.039	3	1.346	11.967*	0.000
Within groups	1.800	16	0.113		
Total	5.839	19			

Table 6

ANOVA for standardization of pH

	Sum of squares	df	Meansquare	F	Probability (p)
Between groups	1.079	13	0.350	24.096*	0.000
Within groups	0.239	16	0.015		
Total	1.318	19			

* Significant at 5% probability

Table 7

Comparison of recovered solids at different temperatures using student's t test

Comparison	Mean Difference	SE	t	Probability (p)
65^0C vs 70^0C	0.8	0.21*	3.81	.002
65^0C vs 75^0C	1.25	0.21*	5.95	.000
65^0C vs 80^0C	0.63	0.21*	3	.009
70^0C vs 75^0C	0.45	0.21*	2.14	.047
70^0C vs 80^0C	0.17	0.21 NS	0.81	.445
75^0C vs 80^0C	0.62	0.21*	2.95	.010

Table 8

Comparison of recovered solids at different pH levels using student's t test

Comparison	Mean Difference	SE	t	Probability (p)
4.0 vs 4.5	0.27	0.08*	3.38	.003
4.0 vs 5.0	0.64	0.08*	8	.000
4.0 vs 5.5	0.22	0.08*	2.75	.012
4.5 vs 5.0	0.37	0.08*	4.63	.000
4.5 vs 5.5	0.05	0.08 NS	0.63	.495
5.0 vs 5.5	0.42	0.08*	5.25	.000

*significant at 5% Probability

NS: Not significant

4. 2. Recovery of solids from SWW

Percentage recovery of solids from SWW by heat coagulation and pH shift with respect to SWW are shown in Table 9. Percentage recovery of solids from SWW by heat coagulation followed by centrifugation was more than that by isoelectric precipitation followed by centrifugation. The observed variation was because of the difference in moisture content (Table 14). Hence percentage recovery of solids from SWW with respect to total SWW was worked out by equating the moisture levels of both the recovered solids to 78.37% which was the moisture content of the solids extracted after heat coagulation. Percentage recovery of solids from SWW with respect to total surimi was worked out on dry weight basis. The data thus generated are summarized in Tables 10 and 11. Statistical analysis of percentage recovery of solids from wash water with respect to both SWW and surimi was carried out using student's t test (Table 12). The results showed that recovery of solids from SWW by heat coagulation was significantly higher than that by isoelectric precipitation. Variations in percentage recovery with respect to SWW are shown in Fig.7 and that with respect to surimi are shown in Fig.8.

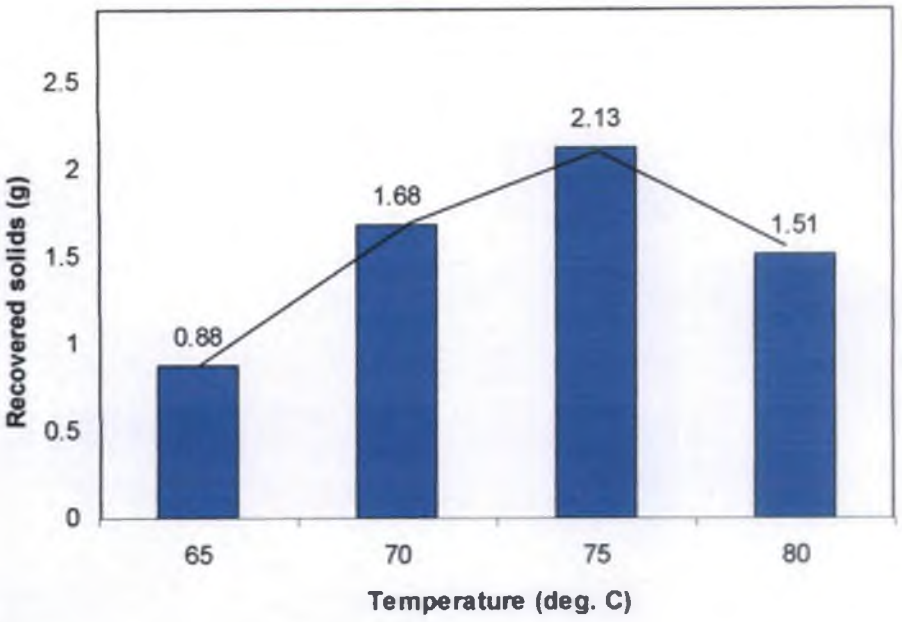

Fig. 5: Effect of temperature on recovery of solids from SWW

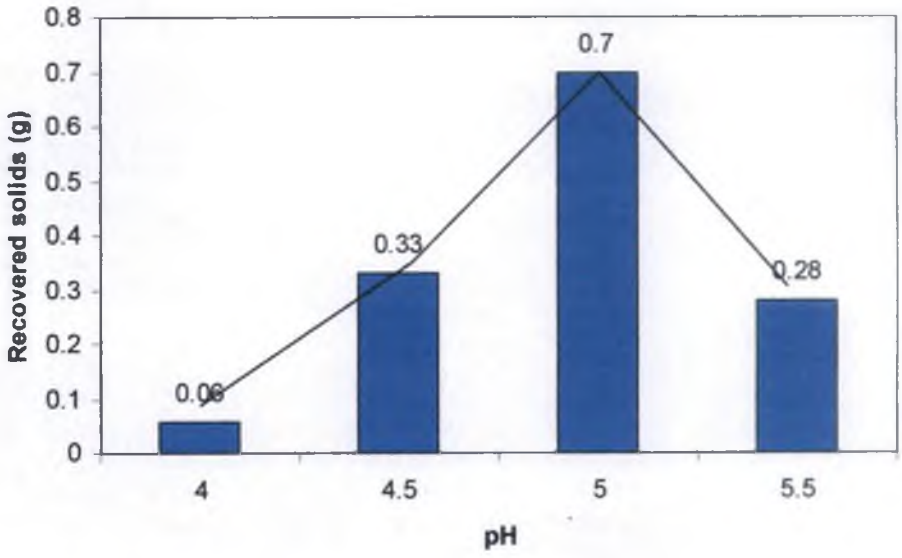

Fig. 6: Effect of pH on recovery of solids from SWW

4. 2. Recovery of solids from SWW

Percentage recovery of solids from SWW by heat coagulation and pH shift with respect to SWW are shown in Table 9. Percentage recovery of solids from SWW by heat coagulation followed by centrifugation was more than that by isoelectric precipitation followed by centrifugation. The observed variation was because of the difference in moisture content (Table 14). Hence percentage recovery of solids from SWW with respect to total SWW was worked out by equating the moisture levels of both the recovered solids to 78.37% which was the moisture content of the solids extracted after heat coagulation. Percentage recovery of solids from SWW with respect to total surimi was worked out on dry weight basis. The data thus generated are summarized in Tables 10 and 11. Statistical analysis of percentage recovery of solids from wash water with respect to both SWW and surimi was carried out using student's t test (Table 12). The results showed that recovery of solids from SWW by heat coagulation was significantly higher than that by isoelectric precipitation. Variations in percentage recovery with respect to SWW are shown in Fig.7 and that with respect to surimi are shown in Fig.8.

Table 9

Percentage recovery of solids with respect to SWW

Variables	No. of observations	Percentage recovery (g/100ml)	
		Mean	SD
Temperature (75^0C)	8	1.97	0.11
pH (5.0)	8	1.17	0.09

Table 10

Percentage recovery of solids with respect to SWW (at constant moisture level)

Variables	No. of observations	Percentage recovery (g/100ml)	
		Mean	SD
Temperature (75^0C)	8	1.97	0.11
pH (5.0)	8	1.41	0.09

Table 11

Percentage recovery of solids with respect to surimi (dry weight basis)

Variables	No. of observations	Percentage recovery (g/100g)	
		Mean	SD
Temperature (75^0C)	8	1.22	0.06)
pH (5.0)	8	0.88	0.06)

Table 12

Analysis of percentage recovery of solids from SWW using student's t test

		Levene's Test for Equality of Variances		t- test for equality of means		
		F	Probability (p)	t	df	Probability (p)
With respect to SWW	Equal variances assumed	.054	.819	11.037*	14	.000
	Equal variances not assumed			11.037	13.635	.000
With respect to surimi	Equal variances assumed	.018	.896	11.136*	14	.000
	Equal variances not assumed			11.136	13.780	.000

*significant at 5% probability

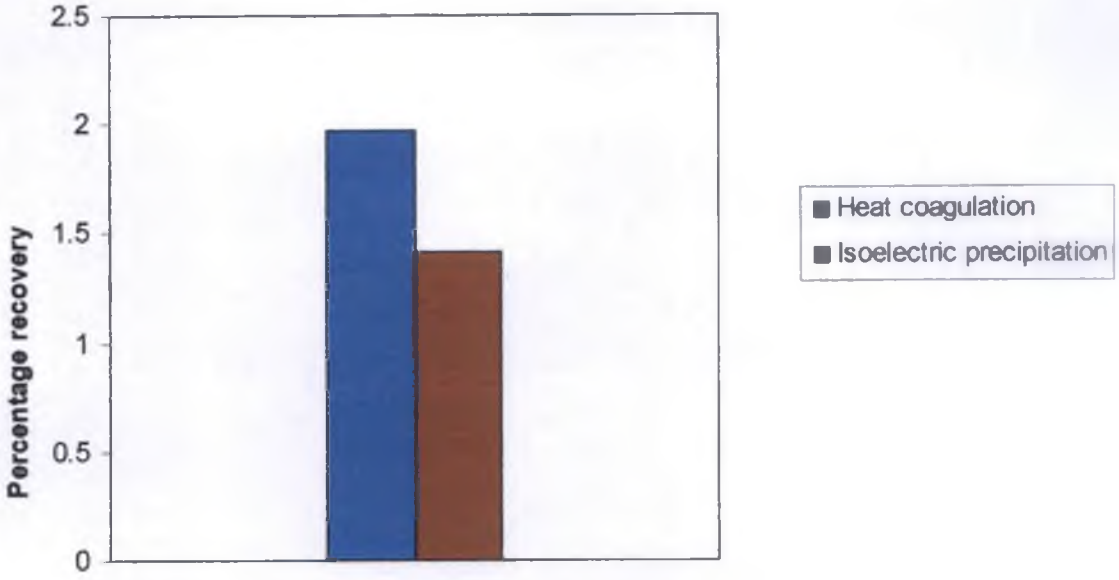

Fig. 7: Percentage recovery of solids with respect to SWW (at constant moisture levels)

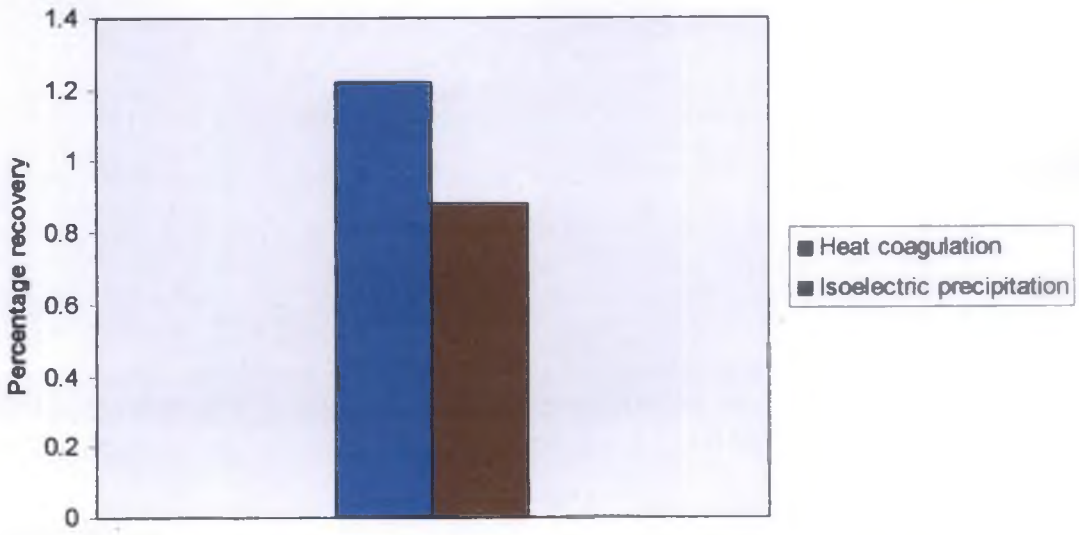

Fig. 8: Percentage recovery of solids with respect to surimi (dry weight basis)

4. 3. Compositional profile

Compositional profile of tilapia meat before and after water leaching is presented in Table 13. Moisture content was found to increase from 75.85 % to 77.56 % upon water leaching whereas crude protein, fat, and ash contents decreased from 21.18 % to 20.16 %, 1.13% to 0.61 % and 1.60 % to 1.37 %, respectively.

Compositional profile of recovered solids by heat coagulation and isoelectric precipitation are given in Table 14. Moisture content of recovered solids by heat coagulation (78.37 %) was higher than that by isoelectric precipitation (74.12 %). But crude protein, crude fat and ash contents were slightly higher for isoelectric precipitated solids at 22.86%, 1.78%, and 1.12%, respectively as compared to 18.86%, 1.41% and 1.08% for heat coagulated solids. Generated data for percentage composition of recovered solids on dry weight basis is given in Table 15. This shows a similar composition for both recovered solids. Variations in percentage composition of recovered solids on dry weight basis is shown in Fig. 9.

Compositional profile of SWW and water after recovery by heat coagulation and isoelectric precipitation are given in Table 16. SWW contains 0.86% protein, 0.16% fat, and 0.14% ash. WR1 contains 0.40% protein, 0.05% fat and 0.086% ash and WR2 contains 0.57 % protein, 0.09 % fat and 0.086 % ash. Statistical analysis of protein, fat and ash percentage in water samples after recovery using student's t test is shown in Table 17. Results showed that protein and fat content were significantly higher in water after recovery by isoelectric precipitation than water after recovery by heat coagulation, but no significant difference was observed for ash content in both water samples. Variations in percentage composition of water samples are shown in Fig.10.

Table 13

Compositional profile of tilapia meat before and after water leaching

Sample	Moisture (%)		Protein (%)		Fat (%)		Ash (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Minced meat	75.85	0.73	21.18	0.86	1.13	0.07	1.6	0.25
Washed meat	77.56	0.94	20.16	1.05	0.61	0.16	1.37	0.17

Table 14

Compositional profile of recovered solids

Sample	Moisture (%)		Protein (%)		Fat (%)		Ash (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
RS1	78.37	1.11	18.86	1.17	1.41	0.20	1.08	0.01
RS2	74.12	0.68	22.86	0.77	1.78	0.20	1.12	0.01

RS1- Recovered solid by heat coagulation followed by centrifugation

RS2- Recovered solid by isoelectric precipitation followed by centrifugation

Table 15

Compositional profile of recovered solids (dry weight basis)

Sample	Protein (%)	Fat (%)	Ash (%)
RS1	87.19	6.52	4.99
RS2	88.33	6.88	4.33

Table 16

Compositional profile of SWW and water after recovery of solids

Sample	Protein (%)		Fat (%)		Ash (%)	
	Mean	SD	Mean	SD	Mean	SD
SWW	0.86	0.03	0.16	0.03	0.14	0.01
WR1	0.40	0.06	0.05	0.01	0.086	0.00
WR2	0.57	0.06	0.09	0.01	0.086	0.01

SWW-Surimi wash water

WR1- Water after recovery by heat coagulation followed by centrifugation

WR2- Water after recovery by isoelectric precipitation followed by centrifugation

Table 17

Analysis of protein, fat, and ash content in water samples after recovery using student's t test

		Levene's Test for Equality of Variances		t- test for equality of means		
		F	Probability (p)	t	df	Probability (p)
Protein	Equal variances assumed	.150	.705	-6.155*	14	.000
	Equal variances not assumed			-6.155	13.970	.000
Fat	Equal variances assumed	.000	1.000	-10.153*	14	.000
	Equal variances not assumed			-10.153	14.000	.000
Ash	Equal variances assumed	1.778	.204	-.210NS	14	.837
	Equal variances not assumed			-.210	12.813	.837

*significant at 5% Probability

NS: Not significant

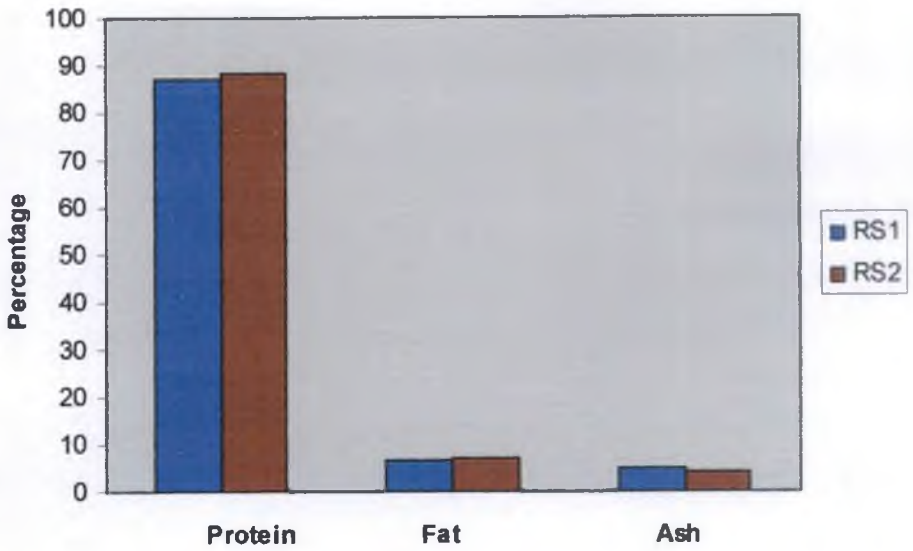

Fig. 9: Percentage composition of recovered solids on dry weight basis

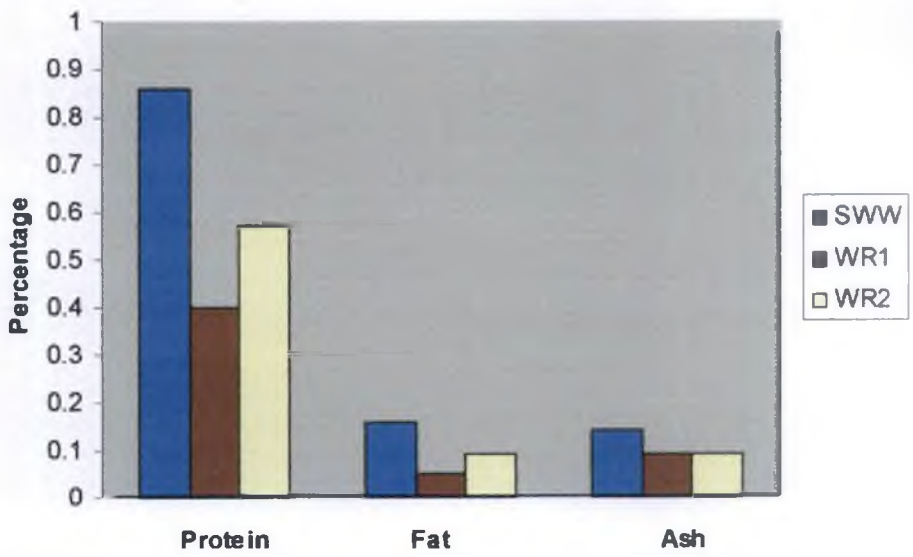

Fig. 10: Percentage composition of water samples

4. 4. Reduction in the composition of water after recovery of solids

Reduction in the composition of water after extraction of solids is given in Table 18. Heat treatment reduced crude protein, crude fat and ash by 53.49%, 68.75% and 38.57%, respectively whereas isoelectric precipitation reduced them by only 33.72%, 43.75% and 38.57%, respectively. Heat coagulation was more effective in reducing protein and fat in SWW than isoelectric precipitation. The effect of the two treatments in reducing protein, fat and ash in the water samples after extraction is given in Fig.11.

4. 5. BOD and COD

Surimi wash water and water samples obtained after extraction of solids were subjected to BOD and COD estimation. BOD and COD values of water samples are given in Table 19. BOD of SWW, water after recovery by heat coagulation and water after recovery by isoelectric precipitation are 5346.88mg/L, 2165mg/L and 2517.5mg/L, respectively. The corresponding COD values are 7220mg/L, 2212.63mg/L and 2606.13mg/L, respectively. Water after recovery by both treatments showed lower BOD and COD than SWW. Water after recovery by heat coagulation showed lower BOD and COD than water after recovery by isoelectric precipitation. The variations in BOD and COD values of water samples are given in Fig. 12. Reduction in BOD and COD by both treatments is given in Table 20. Heat coagulation followed by centrifugation reduced BOD by 59.51% and COD by 69.35%. Isoelectric precipitation followed by centrifugation reduced BOD by 52.92% and COD by 63.90%. The effect of the two treatments in reducing BOD and COD of water after extraction is depicted in Fig. 13. Statistical analysis of BOD and COD of water samples after extraction using student's T test showed that BOD and COD of water samples after extraction by heat coagulation was significantly lower than that by isoelectric precipitation (Table 21)

4. 6. TPC

TPC of minced meat, washed meat and recovered solids are given in Table 22. Recovered solids showed higher TPC than minced meat and washed meat. TPC of SWW and water after recovery are given in Table 23. Water after recovery by heat coagulation followed by centrifugation had lower TPC than water after recovery by isoelectric precipitation followed by centrifugation. Percentage reduction in TPC after extraction is given in Table 24. Heat coagulation reduced TPC of SWW to 93.93% whereas isoelectric precipitation reduced TPC to 56.56%. Variations in TPC of SWW and water after extraction by both treatments are given in Fig. 14.

4. 7. Proximate composition of formulated fish feed

Proximate composition of fish feed formulated using recovered solids as animal protein source was determined and it was compared with a control feed made with clam meat (Table 25). Control feed consisted of 23.13% protein, 6.41% fat, 14.26% ash and 10.28% moisture. Feed prepared using solids recovered by heat coagulation showed 21.33% protein, 6.82% fat, 12.56% ash and 10.22% moisture whereas feed prepared using recovered solids by isoelectric precipitation showed 21.63% protein, 6.91% fat, 12.81% ash, and 10.27% moisture. Results showed that composition of all the three feeds were comparable to each other.

Table 18

Reduction in proximate composition of water after recovery of solids

Sample	Percentage reduction		
	Crude protein	Crude fat	Ash
WR1	53.49	68.75	38.57
WR2	33.72	43.75	38.57

Table 19

BOD and COD of SWW and water after recovery of solids

Sample	BOD (mg/L)	COD (mg/L)
SWW	5346.88	7220
WR1	2165	2212.63
WR2	2517.5	2606.13

Table 20

Reduction in BOD and COD of water after recovery of solids

Sample	Percentage reduction	
	BOD	COD
WR1	59.51	69.35
WR2	52.92	63.90

Table 21

Analysis of BOD and COD of water samples after recovery of solids using student's t test

		Levene's Test for Equality of Variances		t- test for equality of means		
		F	Probability (p)	t	df	Probability (p)
BOD	Equal variances assumed	.600	.452	-2.314*	14	.036
	Equal variances not assumed			-2.314	13.751	.037
COD	Equal variances assumed	.134	.720	-3.585*	14	.003
	Equal variances not assumed			-3.585	13.795	.003

*significant at 5% Probability

Table 22

TPC of minced meat, washed meat and recovered solids

Sample	TPC
Minced meat	8.1×10^3
Washed meat	1.46×10^3
RS1	3.25×10^5
RS2	6.5×10^5

Table 23

TPC of SWW and water after recovery of solids

Sample	TPC (cfu/ml)
SWW	1.22×10^6
WR1	7.4×10^4
WR2	5.3×10^5

Table 24

Reduction in TPC after recovery of solids

Sample	Percentage reduction in TPC
WR1	93.93
WR2	56.56

Table 25

Proximate composition of fish feed

Sample	Protein (%)	Fat (%)	Ash (%)	Moisture (%)
Control	23.13	6.41	14.26	10.28
F1	21.33	6.82	12.56	10.22
F2	21.63	6.91	12.81	10.27

F1- Feed prepared using recovered solids by heat coagulation as protein source

F2- Feed prepared using recovered solids by isoelectric precipitation as protein source

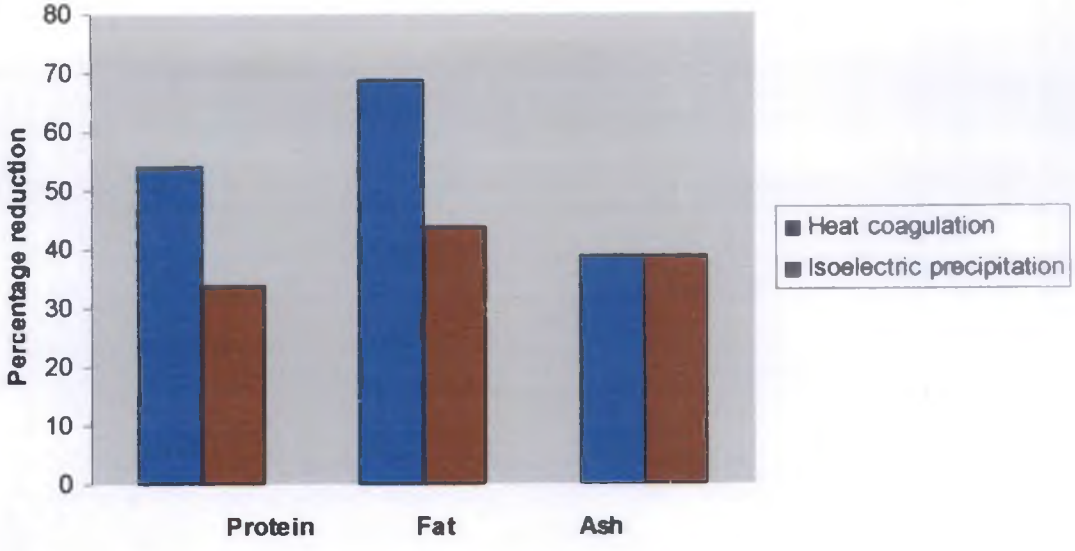

Fig. 11: Reduction in proximate composition of water after recovery of solids

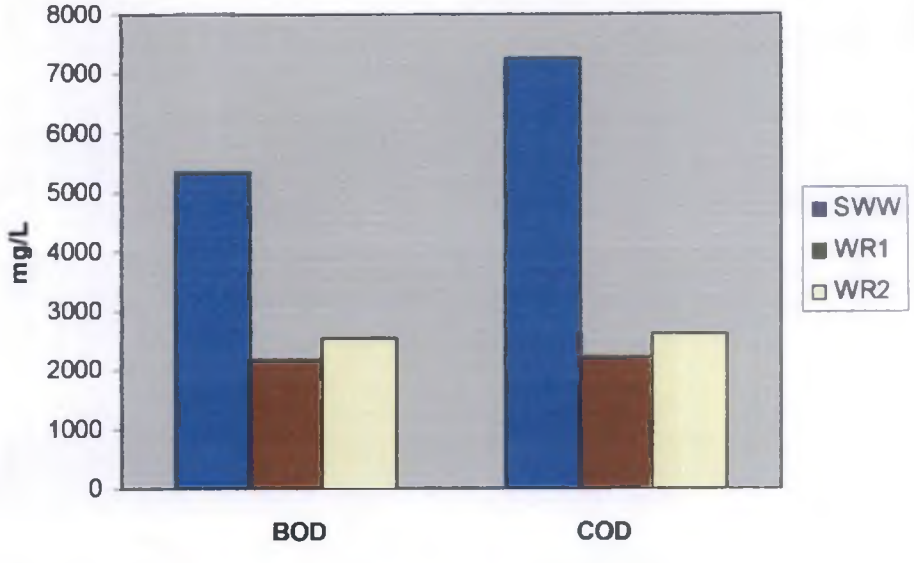

Fig. 12: BOD and COD of SWW and water after recovery of solids

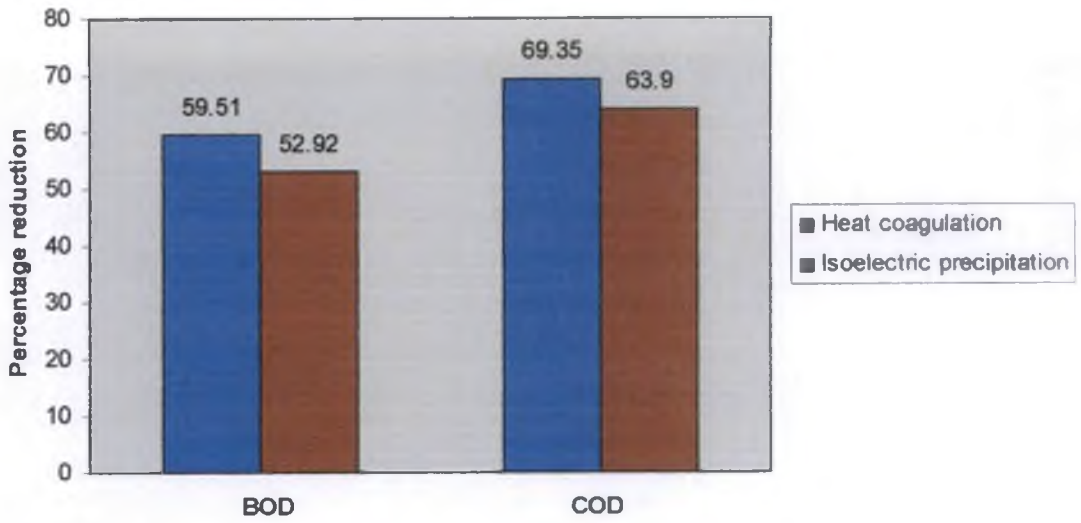

Fig. 13: Reduction in BOD and COD of water after recovery of solids

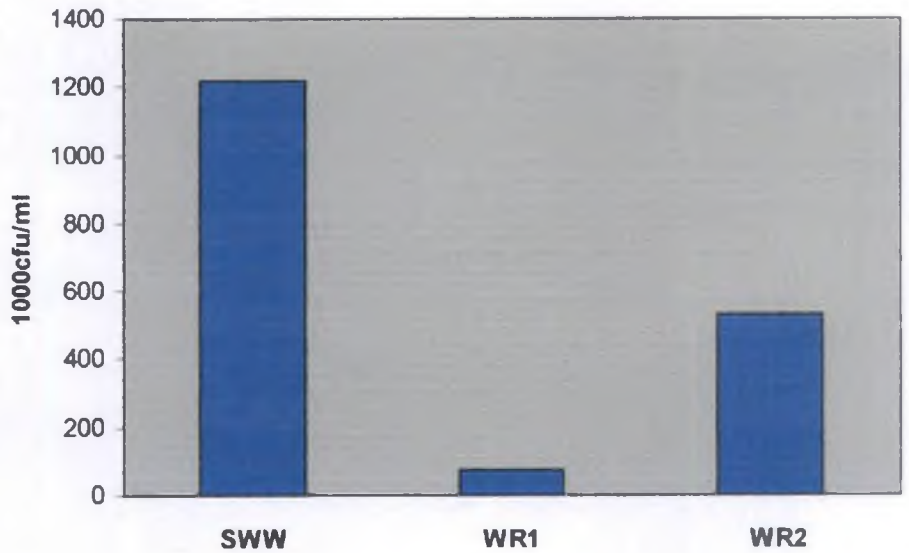

Fig. 14: TPC of SWW and water after recovery of solids

DISCUSSION

5. DISCUSSION

Meat of tilapia *Orechromis mossambicus* was used for the study because of its easy availability. Factory made mince raw material was simulated. The proximate composition of the fish meat (Table 13) indicates that the fish is lean, having only 1.13 % fat. The minced fish was water leached to remove water soluble proteins, pigments, fats etc. Another important purpose of water leaching is to develop sufficient whiteness in surimi. Removal of pigments, fat, water soluble minerals, etc., results in white colour as reported by workers like Kim *et al.* (1996) who did consider it an important index of surimi quality. In this study, 10 volumes water was used for producing surimi, which was relatively less compared to commercial surimi processing. The emphasis here was more on SWW rather than surimi. Recycling of surimi processing water is gaining in importance in this context due to rising utility costs, limited water resources and pollution problems associated with recovery.

Different temperature for heat coagulation were tried based on the work of Kanjanapongkul *et al.* (2008) who developed a batch type ohmic heater to investigate the reduction of solids contained in threadfin bream SWW. They optimized the temperature for heat coagulation at 70^0C. A water bath was used in the present study rather than ohmic heating as in their case. Isoelectric precipitation was selected with pH as a variable considering the fact that isoelectric pH of fish is in the range of 5 to 6. Hydrochloric acid of 10% concentration was used for isoelectric precipitation because of the effectiveness of inorganic acids in lowering pH. With increase in temperature, percentage recovery of solids increased, reached a maximum at 75^0C, and then decreased at 80^0C. This trend differed from the findings of Kanjanapongkul (2008), who observed an increase in recovery with increase in temperature. Nishioka and Shimizu (1983) also reported similar results on the effect of temperature on recovery upon washing of minced fish meat. Scopes (1994) also showed that higher temperatures created more denaturation than lower temperatures. In the

present study recovery was seen decreasing at 80^0C, perhaps because of the use of water bath rather than ohmic heating for heat coagulation. Use of a water bath had resulted in increased adherence to the surface at 80^0C leading to lesser recovery. Thus optimum temperature for heat coagulation was selected as 75^0C. Huang *et al.*, 2007 observed that holding the samples at constant temperature for longer periods does not improve solid removal, hinting that it is the temperature that is important and not the period of holding which may only improve the quantum of heat and not coagulation.

While standardizing the pH for recovery maximum recovery was obtained when SWW was subjected to a pH of 5.0. Dewitt and Morrissey (2002) optimized pH as 4.5, with additional heat which recovered protease in active form. In the present study isoelectric precipitation was done without heating and recover of enzymes was not an objective. Optimum pH which satisfies maximum recovery falls in the range 3.5 to 5.2 which is the isoelectric range of water soluble muscle proteins as reported by Chen *et al.* (2003). In the present study maximum recovery was obtained at the isoelectric pH of 5.0 which was chosen as the optimum pH. Statistical analysis showed a significant difference in solid recovery at varying temperatures and varying pH. Solubility is considered as one of the functional properties of proteins, hence the loss of solubility could be taken as a criterion of protein denaturation (Wolf, 1970 and Wu and Inglet, 1974). The solubility of the recovered proteins decrease with decreasing pH. Minimum solubility is seen at the isoelectric pH. As the pH approaches isoelectric point, electrostatic repulsion between the individual molecules no longer exists which results in precipitation (Harris and Angal, 1989). Reaction time of the pH shift at a particular temperature had little or no effect on the percentage of precipitation of proteins from SWW (Scopes, 1994). Dewitt and Morrissey (2002) reported that the type of acid applied did not significantly affect the protein content of water after isoelectric precipitation.

Moisture levels in both recovered solids were different and hence the percentage recovery with respect to SWW was worked out after equating the moisture content for both the recovered solids to 78.37% which was the value shown by recovered solids by heat coagulation. Percentage recovery with respect to surimi was also worked out on dry weight basis. Percentage recovery of solids from SWW by heat coagulation followed by centrifugation with respect to both SWW and surimi were found to be higher than that of isoelectric precipitation. Hence it was concluded that heat coagulation was more efficient in recovering solids from SWW than isoelectric precipitation. This is in correlation with the works of Dewitt and Morrissey (2002).

Compositional profile of tilapia mince after water leaching showed an increase in moisture level than mince before water leaching. Protein content decreased on water leaching, which may be primarily due to the loss of water soluble proteins during the leaching process. For the same reason, fat content and mineral content had also decreased. Sijo *et al.* (2002) attributed the reduction in ash content to the removal of water soluble mineral constituents from meat. An increase of 1.71% in moisture content was noticed probably due to the hydration of myofibrillar proteins. Similar results have been reported by Suvanich *et al.* (2000). Many workers (Suzuki, 1981; Hultin, 2002; Park, 2005) have recommended water leaching to the extent of two to three times for lean meat with a last wash using dilute sodium chloride solution in order to prevent excessive water absorption. The same method was adopted in the present study. However, a slightly high hydration of proteins seemed to have taken place.

Moisture content of recovered solids by heat coagulation was found to be higher than that of isoelectric precipitated solids. All samples were dried under similar conditions. However the quantity of recovered solids was small for isoelectric precipitation. Because of increased moisture content in the solids recovered by heat coagulation, its protein, fat and ash contents were lower than those for isoelectric precipitated solids. But the percentage composition genarated

on dry weight basis (Table 15) shows a comparable composition in both recovered solids.

Compositional profile of water samples was also determined. Surimi wash water protein comes in the range, 0.5 to 2.3% as suggested by Lin and Park, 1996, Park and Morrissey, 2000, Morrissey *et al.*, 2000, and Savant and Torres, 2003. Here SWW was obtained by mixing all the wash waters with the press water, contrary to earlier works where wash waters of each step was analyzed separately. Compositional profile of SWW was similar to that obtained by Lin *et al.* (1995). They had used a dehydrator for the experiment. Hence protein content in the first washing was found to be comparatively higher than what was obtained here. All other washings showed similar composition. Comparison was made by taking average data for all washings. Similarity in average protein concentration in SWW was obtained when all washes excluding first washing was considered. Also here commercial surimi manufacturing process was only simulated. In the present study only small quantities were processed. The extent of comminution was less resulting in reduced loss of myofibrillar proteins than what usually happens during commercial surimi production. But fat and ash levels compare well with the results reported by earlier workers. Variations were observed with the studies of Raweewan *et al.* (2004), since they analyzed SWW after prefiltration using Whatman No. 4 filter paper. Protein, fat and ash content of SWW was reduced to 46.51%, 31.25% and 61.43% respectively after heat coagulation followed by centrifugation whereas isoelectric precipitation followed by centrifugation reduced them to only 66.28%, 56.25%, and 61.43% respectively. It was observed that percentage reduction in crude protein and crude fat by heat coagulation was higher (Table 18) as compared to that for isoelectric precipitation. Statistical analysis also proved that crude protein and crude fat content in water after recovery by heat coagulation was significantly lower as compared to the corresponding values for isoelectric precipitation. But percentage reduction in ash level by both treatments was observed to be same. Difference in protein reduction may be due to increased coagulating effect of

temperature than pH. Also heat causes melting of fat resulting in its easier removal. We can also infer that heat and pH do not have any particular role in removing minerals. Reduction, if any, could be due to the interaction with the coagulated protein and fat. Effect of temperature in protein removal from SWW had been highlighted by Kanjanapongkul *et al.* (2008) who developed a batch ohmic heater to investigate reduction of solids contained in threadfin bream SWW. Small variations found in the present work may be due to the type of heating adopted for SWW. Effect of pH in protein removal from SWW is supported by the studies of Dewitt and Morrissey (2002).

. BOD and COD of water samples were analyzed. The extensive washing of minced fish flesh caused a high loss of organic substances which resulted in extreamely high BOD and COD in waste water (Table19) compared to 900 to 1, 200 mg/L from poultry processing waste (Chang *et al.*, 1989). COD levels of SWW were comparable with the findings of Lin *et al.* (1995). They reported an 89 to 94% reduction in COD values by applying ultrafiltration. This suggests the use of ultrafiltration for improving quality of SWW. BOD and COD levels of SWW obtained in this study fell in the lower limit suggested by workers. This may be due to the variation in the extent of mincing of fish meat, relative to commercial mincing operations. BOD and COD of SWW were in correlation with the observations of Raweewan *et al.* (2004). But they obtained BOD and COD for each washing separately. But in this present study wash waters and press water were collected and mixed together and was used for analysis. Comparison was made by taking the average BOD and COD values for all washings. Heat coagulation reduced BOD of water after recovery to 40.49%. A higher reduction in BOD was observed by Kanjanapongkul *et al.* (2008). These variations may be due to the two different types of heat treatment adopted. Isoelectric precipitation reduced BOD of water after recovery to 47.08%. Statistical analysis of BOD of water samples after recovery showed that heat coagulation was more effective in reducing BOD than isoelectric precipitation. COD of water after recovery by heat coagulation and isoelectric precipitation were 30.65% and 36.1%, respectively.

Statistical analysis of COD of water samples after recovery also showed that heat coagulation was effective in reducing COD than isoelectric precipitation. Better reduction in both BOD and COD for heat coagulation was due to efficiency of heat in reducing crude protein and crude fat content as compared to that of pH.

TPC of water samples were determined. Heat coagulation reduced TPC of SWW almost 100 times whereas isoelectric precipitation reduced TPC only by about 50 times. This variation may be due to the killing effect of heat on microorganisms.

Analysis of proximate composition of fish feed showed that both control and prepared feeds showed a similar composition. This suggests the use of these recovered solids in fish feed as animal protein source. This cheap source of proteins that generally goes to plants' waste stream could effectively replace other animal protein sources that are costly.

Works were done with ultrafiltration and microfiltration for the recovery of enzymes, mainly proteases. In this study, main objective was to recover solids and to incorporate these solids in animal feed as animal protein source. Since ultrfiltration and microfiltration are costly it is not feasible for the industry to implement these techniques for treating their wastes. Also because of large quantity of SWW, recovery using these methods will be cumbersome. A combination of heat and ultrafiltration was tried by Dewitt and Morrissey (2002) in recovering proteases.

This study suggests surimi industry, two methods that are comparatively cheap and easy to implement for recovering solids from SWW. The results obtained showed that the nutrient load in SWW could not be removed completely, which was responsible for BOD and COD. Ultrfiltration and microfiltration can recover these solids remaining, but since the use of heat and pH inactivated the

enzymes, it will not be feasible for the industry to use these techniques for further recovery.

From the foregoing work it is concluded that both heat coagulation and isoelectric precipitation are effective in recovering solids from SWW and in improving its quality. However heat coagulation is the better of the two. Recovery of solids can render the effluent much safer to discharge into water bodies. The recovered proteins can be used to replace other costly animal protein sources in formulating fish feed.

SUMMARY

6. SUMMARY

1. Recovery of solids from surimi wash water (SWW) obtained during water leaching of fish mince, was studied. Two techniques namely heat coagulation and isoelectric precipitation were tried. The efficiency of two techniques in solid recovery was evaluated. The recovery of solids also helped in substantially reducing the effects of eutrophication in the effluent (SWW) discharge. The recovered solids were used as an animal protein source to prepare a fish feed.

2. Fresh Tilapia (*Orechromis mossambicus*) was used as raw material for the study and was transported from Puthuveypu to the laboratory chilled in ice.

3. Meat was picked , minced strained, water leached with three times water in three cycles, dewatered and screw pressed. All washes and press waters were collected and mixed together. This water termed surimi wash water (SWW) was used for the study.

4. The study was done after standardization. Four different temperatures viz., 65^0C, 70^0C, 75^0C, and 80^0C and four different pH levels of 4.0, 4.5, 5.0 and 5.5 were studied to establish conditions for maximum recovery. Optimum temperature thus standardized was 75^0C and the optimum pH was 5.0.

5. Recovery was done using optimum temperature and optimum pH. **Percentage recovery** for each variable was determined with respect to SWW as well as surimi. Heat coagulation showed maximum recovery than isoelectric precipitation.

6. Comparison of proximate **composition of recovered solids** was done. Solids recovered by both techniques showed similar composition.

7. Proximate **composition of water samples** before and after recovery was determined. Comparison of proximate composition of SWW and water after extraction was done. SWW showed higher composition followed by water after recovery by isoelectric precipitation. **Percentage reduction** in composition was determined and it was found that heat coagulation was more effective in reducing the composition than isoelectric precipitation.

8. **BOD and COD** of water samples were determined. SWW showed more BOD and COD followed by water after recovery by isoelectric precipitation. Heat coagulation was better than isoelectric precipitation in reducing both.

9. **TPC of water samples** were also determined. Water samples subjected to heat coagulation showed a lower TPC than the other two samples. Maximum TPC was shown by SWW.

10. **Proximate composition of fish feed** prepared with the recovered solids as animal protein source was determined and was compared with a control feed prepared with clam meat. It was found that protein, fat and ash levels were comparable in all the three feeds. Recovered solids could be considered as an important animal protein source while preparing fish feeds.

11. Heat coagulation and isoelectric precipitation are two effective means of recovering solids from SWW, with **heat coagulation ranking first**. BOD, COD, TPC, and proximate composition of SWW samples after recovery showed substantial reduction in their levels suggesting the effect of heat coagulation and isoelectric precipitation in **reconditioning water**. The study also recommends to feed industry, **a cheap animal protein source** for preparing fish feed.

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ABSTRACT

ABSTRACT

A study was conducted aimed at testing the efficiency of pH reduction and heat coagulation in the recovery of solids from surimi wash water (SWW) generated during water leaching of the meat of tilapia (*Oreochromis mossambicus*) and to reduce the nutrient load in SWW. The study also included the preparation of a fish feed with the recovered solids.

Temperature for heat coagulation was optimized among four different temperatures viz., 65°C, 70°C, 75°C and 80°C. Optimum temperature that provided maximum recovery was 75 °C. Isoelectric precipitation was optimized using four different pH levels viz., 4.0, 4.5, 5.0, and 5.5. Optimum pH which yielded maximum recovery was pH 5.0. By heat coagulation 1.97% solids with respect to SWW was recovered whereas isoelectric precipitation yielded only 1.41% solids. In relation to surimi the yield was 1.22% and 0.88% respectively. Heat coagulation reduced crude protein, crude fat and ash of SWW by 53.49%, 68.75% and 38.57% respectively whereas isoelectric precipitation reduced these parameters by 33.72%, 43.75% and 38.57% respectively. Heat coagulation reduced BOD and COD of SWW by 59.51% and 69.35% respectively whereas isoelectric precipitation reduced their levels by 52.92% and 63.9% respectively. Analysis of proximate composition of fish feed showed that the control using clam meat and the two feeds using recovered solids showed similar composition. Thus the use of these recovered solids in fish feed as an animal protein source is a possibility.

This study recommends to surimi industry, two methods that are comparatively cheap and easy to implement for recovering solids from SWW. Heat coagulation and isoelectric precipitation can effectively recover solids from SWW and improve its quality. However, heat coagulation is the more efficient method of the two. After solid recovery, the wash water effluents are rendered

safer. The recovered solids can serve as a good substitute to clam meat in the fish feed preparation even though the quantum of proteins recovered are relatively small.