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Effect of pH-shift processing on biochemical and functional properties of surimi extracted from pink perch *Nemipterus japonicus* (Bloch, 1791)

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Abstract
The pH-shift processing involves the process of solubilization of muscle protein at high alkaline pH (11.0) and low acidic pH (3.0) while precipitation at pH (5.5) of fish muscle. This method of processing was used for the preparation of surimi from the minced obtained from pink perch, *Nemipterus japonicus* (Bloch, 1791). The study compared biochemical and functional properties of surimi prepared by conventional and pH-shift processing (alkaline and acid) solubilization, along with change in characteristics, if any, during frozen storage. The highest protein recovery rate (%) was obtained in alkaline (76.12%) method, followed by (58%) and conventional method (54.57%). With regard to the proximate composition, highest moisture content was observed in acid surimi, highest protein content in conventional surimi. Lipid and ash content were low in all the surimi samples. The protein solubility and total -SH groups were highest in conventional surimi followed by alkaline-aided and acid-aided surimi. The initial Ca^{2+} ATPase activity of NAM was in the order of conventional > alkaline > acid which decreased during frozen storage. The gel strength of conventional surimi was observed to be 255.56 g.cm which was significantly higher than alkaline-aided and acid-aided surimi which continues to decrease in frozen storage. The whiteness value was also significantly higher in conventional surimi than other two samples. The expressible moisture content has shown decreasing trend during frozen storage. The SDS-PAGE of the sample proteins remains unaffected during frozen storage with molecular weight approx. 210 kDa and the actin with a molecular weight of 45 kDa, however, appearance of myosin heavy chain (MHC) was replaced by light chain (25 kDa) during frozen storage.

Keywords: pH-shift processing, surimi and functional properties

Introduction
Fish has always been an important source protein and other nutrients for human. As the global, the production of capture and aquaculture fisheries was 178.5 million tonnes and out of that 156.4 million tonnes utilized for human consumption (FAO, 2020) [1]. Fish processing by-products, small fatty fishes and by-catch protein sources were underutilized by the human consumption. The main challenge facing by the fisheries was to find an economic way to convert these underutilized fish protein resources into human food to meet demand (Kristinsson *et al*., 2006) [51]. Production of mince as a new technology assumes importance when applied to low value fish (Balachandran, 2001) [3] and by-catch which face difficulty in marketing as fresh fish or processing into conventional product.
Suzuki (1981) [4], defines surimi as fish meat is minced and water-washed, and after anti-denaturants was added, frozen into a block form and this technology was broadly developed. Surimi is produced by repeatedly washing of mechanically separated fish flesh with chilled water (5^0C) until most of the water-soluble proteins were removed. As stated by Lanier and Lee (1992) [5], the washing process has its own importance for maintaining quality of surimi because it removes fat and undesirable materials (blood, pigments and odorous substances) and increases the concentration of myofibrillar protein, thereby improving gel-forming ability. Textural properties of muscle protein-based gel and emulsion type comminuted products depends on the myofibrillar proteins (Fukazawa *et al*., 1961) [6]. In the developing countries the popularity and demand for surimi and surimi-based products shows an increasing trend. The frozen surimi is converted to a variety of products in shore-based factories.
The major aim of conventional and pH-shift method used in surimi production is to increase the concentration of myofibrillar proteins by elimination of unwanted substances. Methodologically, the major difference has been noticed in washing process.

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In the previous method, repeated washing is carried out while in the latter, alternate acid and alkaline wash is given. According to the research studies, Hultin and Kelleher, 2000 [7] stated that pH-shift method gives higher percentage protein than conventional method. So, pH-Shift processing can be considered as a potential alternative to conventional process of surimi processing.

Proteins recovery using acid or alkali extraction followed by isoelectric precipitation provides extremely high yields (35–45%) with the inclusion of sarcoplasmic proteins and it also demonstrates better functional properties. The extraction mechanism of these two processes is to dissolve muscle protein at low or high pH by centrifugation to separate soluble protein, bone, skin, connective tissue, cell membrane and neutral lipid. Collect and recover the solubilized protein by isoelectric precipitation to provide a functional and stable fish protein isolate (FPI) (Kristinsson et al., 2006) [51].

As proteins take on more positive or negative net charges, they gradually begin to interact electrostatically with water (i.e., protein–water interactions). An inverse relation exists between protein-water and protein-protein interaction. Therefore, as the protein molecules become charged, more water associates on and around the protein surface and the proteins become water-soluble. However, the pH of the protein solution can be adjusted so that the number of negative charges on the protein surface is equal to the number of positive charges. Therefore, protein molecules assume zero static charge. The net electrostatic charge of a protein at pH is equal to zero is called the isoelectric point (pI) (Gehring et al., 2011) [8]. Using pacific whiting fillets, a conventional three-washing cycle surimi processing yielded only 40% recovery compared with 60% recovery using acid-aided processing (Choi and Park, 2002) [9].

The functionality of food protein refers to the physical and chemical properties that affect the performance of protein in the food system during processing, storage, preparation and consumption, that control protein function include size; shape; amino acid composition and sequence; net charge and distribution charge; hydrophobicity/hydrophilicity ratio; secondary tertiary and quaternary structure; molecular flexibility/rigidity; and other component interaction/reaction ability. Since proteins have multiple physical and chemical properties, it is difficult to determine the role of each property regard to a given functional property (Damodaran et al., 1996) [10].

In India, among the several marine fish species, threadfin bream (Nemipterus japonicus) was considered as important fish species for surimi production due to its availability, low cost and white meat (Singh and Balange, 2005). It belongs to the family Nemipteridae and more than 10 species are commonly found in the Indo-West Pacific region in tropical and sub-tropical waters. The fish forms an important part of the trawl catch with the greater catches being landed in Thailand, India, Vietnam, Indonesia, The Philippines, and Malaysia. Threadfin bream is the main species (70%) used for surimi production and various other species are also used that include lizard fish, croaker, big eye snapper, goat fish, ribbon fish, and sardine (Park, 2013) [12]. India is reported to process and export about more than 1.0 lakh metric tonnes of surimi annually. Therefore, the processing technology for preparation of surimi as assumed immense importance.

In the present studies, biochemical and functional properties of fish muscle proteins obtained from conventional and pH-shift processing (alkaline and acid solubilization) in order to reduce consumption of water by minimizing the waste washing cycles were studied.

Material and Methods
Materials
Fish
Pink perch (Nemipterus japonicus) was purchased from Mirkarwada landing centre, Ratnagiri and brought in iced (2^0C) condition to College of Fisheries, Shirgoan, Ratnagiri.

Methods
Preparation of surimi
pH-shift processing. Mince was separated using meat mincer. The process of acid/alkali solubilization was performed as per the protocol given by Hultin and Kelleher (2000) [7].

Preparation of surimi by conventional method. The conventional surimi was prepared according to the method given by Chaijan et al. (2010) [13] with slight modifications.

Proximate Composition
Moisture content, protein, lipid and ash content of conventional surimi was determined as per standard methods (AOAC, 2005) [14].

Protein recovery
Protein recovery of the washed mince from different washing methods was determined according to the method of Kim et al. (2003) [15]. The recovery of protein was calculated as follows:

$$\text{Protein recovery (\%)} = \frac{\text{weight of recovered washed mince (g)}}{\text{weight of initial minced sample (g)}} \times 100$$

Protein Solubility
Protein solubility was determined using method of Choi and Park. (2002) [9]. Protein solubility (%) was defined as the fraction of the protein remaining soluble after centrifugation and calculated as follows:

$$\text{Protein solubility (\%)} = \frac{\text{protein concentration in supernatant}}{\text{protein concentraion in homogenate}} \times 100$$

Total sulfhydryl (SH) groups
The total SH groups of myofibrillar protein fraction were estimated according to the method given by Sedlak and Lindsay (1968) [16].

Natural actomyosin (NAM) preparation
Extraction of natural actomyosin from surimi was done according to the method described by Benjakul et al. (1997) [17].

Assay of ATPase activity
The Ca^{2+}ATPase assay of actomyosin was estimated according to the method of MacDonald and Lanier (1994) [18].

Estimation of inorganic phosphate
Inorganic phosphates were estimated by the method of Fiske and Subbarow (1925) [19].

Surimi gel preparation
The heat-induced surimi gel was prepared according to the method given by Kunimoto et al. (2016) [20].

Gel strength of surimi gel

The gel strength of surimi gel was measured by a texture analyser. Chill stored gels were equilibrated and tested at room temperature. Five cylinder-shaped samples of 2.5 cm in length were prepared from each gel. The breaking force (gel strength) and deformation (elasticity or cohesiveness) were measured by using the texture analyser equipped with a cylindrical plunger (5 mm diameter; 60 mm per min penetration speed). Measurements were taken in triplicates.

Whiteness measurement of surimi gel

Whiteness of surimi gels was measured using a Hunterlab Miniscan EZ Model No. 4500L. The measurement of *L**(lightness), *a** (redness/green) and *b** (yellowness/blueness) was performed in five replications. The following formula was used to calculate the whiteness Park (1994) as follows.

Whiteness = 100 - [(100-L*)^2 + a^{*2} + b^{*2}] $^{1/2}$

Expressible Moisture of surimi gel

Expressible moisture content of surimi gel samples was measured according to the method of Benjakul *et al*. (2001) [22]. Calculate the expressible moisture content by using the following equation:

Expressible moisture content (%) = 100[(X-Y)/X]

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoretic analysis of protein using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli (1970) [23] using 5% stacking gel and 8% separating gel.

Result and Discussion

Recovery / yield of proteins

In the present investigation, highest recovery/ yield of protein was obtained in alkaline method followed by acid (58%) and conventional (54.57%) method (table 1). Kristinsson and Ingadottir (2006) [51] recorded highest yield (61 to 68%) from alkaline than acid (56 to 61%) method from tilapia (*O. niloticus*). Bastista (1999) [25] also claimed highest yield (80.6% to 62.9%) by alkaline method, after the isoelectric precipitation of hake (*Merluccius merluccius*) and monkfish (*Lophius piscatorius*) respectively, modifying slight difference in processes of Hultin and Kelleher, some salts was added and extend the extraction time and higher temperatures were used. Using the acid and alkaline methods, Undeland *et al*. (2002) [26] found protein yields of 74±4.8% and 68±4.4% respectively, from white muscle of herring (*Clupea harengus*), the lower yield of alkaline due to a larger sediment's formation in first centrifugation. Rawdkuen *et al*. (2009) [27] found highest recovery by the acid method (85.4%) followed by alkaline (71.5%) and conventional method (67.9%) from tilapia muscle. The increase in yield acid and alkaline methods was probably due to recovery of sarcoplasmic proteins in muscle. The higher recovery of proteins in acid/alkali solubilization can also be attributed to the minimum solubility of proteins that occurs at above isoelectric pH of proteins which usually lower than acid and alkaline pH values. As the majority of food proteins are acidic (pH > 7), they have minimum solubility at isoelectric pH (4.0-5.0) and maximum solubility at alkaline pH. The minimum solubility at isoelectric pH is mainly due to lack of electrostatic repulsion which promotes aggregation and

precipitation via hydrophobic attractions. Shabanpour *et al*. (2015) [28] reported that the higher yield from alkaline method (61% to 68%) from silver carp (*Hypophtalmichthys molitrix*) comparing to acid method (56% to 61%) and there was a statistical difference sample (*p*<0.05). The observation made with present study regard to yield proteins was in agreement with Kristinsson and Ingadottir (2006) [51] and Batista (1999) [25]. The higher recovery of protein was obtained from alkaline method and lower from acid method was due to precipitation of sarcoplasmic protein as well.

Table 1: Protein recovery by conventional, alkaline/ acid solubilisation method.

Surimi preparation methods	pH of protein solubilisation	Protein recovery (%)
Conventional Surimi	7.0	54.57
Alkaline surimi	11.0	76.12
Acid surimi	3.0	58

Proximate composition of surimi

As shown in table 2, the proximate composition of all surimi samples, moisture content of acid surimi (80.20±0.49%) was higher followed by alkaline (79.06±2.43) and conventional (77.97±1.96%) surimi. The protein content was found to be higher in conventional surimi (20.33±0.68) comparing to acid and alkaline surimi, which is mainly due to charged group in alkaline and acid (ion-dipole interactions. The lipid and ash content were relatively lower in all the surimi samples. Undeland *et al*. (2002) [26] found that protein isolates samples prepared from alkaline method were lower in fat than the acid solubilized method. Thus, from the present study, it is evident that alkaline method is effective for removal of fats. Chen and Jaczynski (2007) [29] reported lipid removal from the recovered proteins is often desirable because the lipids of fish are easily oxidized, leading to development of rancidity, usually related to fishy smell. Results of the present study showed that ash content in acid surimi was lower than the alkaline surimi. Marmon and Undeland, (2010) [30] reported that the amount of ash should be seen as a measure of impurities, and of great importance to reduce it to concentrate the proteins.

Table 2: Proximate composition of conventional surimi, alkaline / acid surimi.

	Moisture (%)	Protein (%)	Lipids (%)	Ash (%)
Conventional Surimi	77.97±1.96	20.33±0.68	0.21±0.03	1.47±0.03
Alkaline Surimi	79.06±2.43	18.22±0.02	0.52±0.03	2.20±0.22
Acid Surimi	80.20±0.49	17.71±0.32	1.03±0.01	1.38±0.03

Protein solubility

The results of the study showed in table 3, the protein solubility due acid/alkali treatment was affected the surimi samples. Higher solubility values were observed in conventional surimi (68.34%) followed by alkaline surimi (64.43%) and acid surimi (60.92%). Inverse relationship was observed between storage time and protein solubility. Shabanpour *et al*. (2015) [28] reported that the highest protein solubility in common carp and silver carp was found in conventional surimi (0.89 mg/g, 0.94 mg/g), followed by the alkaline-aided process (0.38 mg/g, 0.86 mg/g) and acid-aided process (0.40 mg/g, 0.24 mg/g), it indicates that conventional

surimi gives higher protein solubility. Protein solubility in fish muscle has been used as a criterion for the alteration of proteins and decrease in protein solubility, as a result of protein denaturation, subsequently increased hydrophobic interactions, which caused precipitation of the protein (Zayas, 1997) [31]. Shabanpour *et al*. (2016) [32] reported solubility of carp surimi obtained using method of acid and alkaline process was lower than the conventional methods. In common carp (*C. carpio*) a significant difference in protein solubility was observed between treatments until the end of the frozen storage ($p<0.05$). During early stage of freeze denaturation of fish proteins, both myosin and actin apparently form an insoluble fraction that accounts for the observed decrease in

protein solubility (Jiang and Lee, 1985) [33]. During freezing and frozen storage, the denaturation and aggregation of protein started from formation of disulphide bonds, and rearrange the hydrophobic and hydrogen bond regions on an intra and intermolecular basis (Buttkus, 1974) [34]. From the present results, the decrease in protein solubility was observed during the frozen storage at -18 ^{0}C for 4 months which was due the denaturation of protein caused by freezing and the interaction of disulphide or hydrophobic interactions might be a major contributor for the changes. The decrease in solubility values of surimi protein solubility during frozen storage may due to result of formation ice.

Table 3: Changes in protein solubility (%) of pink perch surimi prepared by conventional method and acid-alkaline solubilization method during frozen storage.

	Protein solubility (%) of surimi during frozen storage (months)				
	0	1	2	3	4
Conventional surimi	68.34±4.94[1]	66.73±4.29[1]	66.14±4.61[1]	52.75±2.35[1]	43.04±4.50[1]
Alkaline surimi	64.43±4.92[1]	59.19±3.77[2]	58.31±5.55[1]	47.90±3.91[1]	41.90±2.77[1]
Acid surimi	60.92±3.76[1]	54.54±5.96[2]	43.92±3.67[2]	38.83±3.48[2]	36.10±4.14[1]

Values are mean±SD. n=3. Values in the same column with different superscripts are significantly different ($p<0.05$)

Total sulfhydryl –SH groups

As showed in table 4, the total sulfhydryl groups of all the samples during frozen storage showed decreasing trend. While reduction was more in pH-shift processing (acid and alkali-aided surimi) as compared to conventional surimi during frozen storage months. The decrease in total sulfhydryl content due to formation of disulfide bonds or oxidation of sulfhydryl groups or exchange of disulfide bond (Benjakul *et al*, 1997) [17]. Yogsawatdigul and Park (2004) [35] reported decrease in SH groups in acid treated samples, suggesting that oxidation and SH/S-S interchange reactions could occur during acid solubilization. Nurkhoeriyati *et al*. (2011) [36] suggested that SH-groups become more reactive on fish protein isolates (FPI) made from alkali treatment compared to FPI prepared from acid treatment. Therefore, the values of SH groups in the acid treated sample were the least compared to conventional and alkali. This inter change reaction i.e

oxidation of SH groups to disulphide bond can occur within a single denature protein if it contains free SH groups and disulphide bond. The interchange reaction often leads to a decrease in stability of protein molecule. Thawornchinsombut and Park (2006) [37] found that, under the alkaline conditions, the SH content significantly decreased as pH increased. This result perhaps indicated that SH groups became more susceptible to oxidation at alkaline pH resulting in the formation of disulfide bonds. From the first month to the third month a gradually increasing process was observed which could be due to the opening of the protein structure (Li-schen *et al*. 1985) [38] and this process again decreased from the third month to the fourth month. From the results, it was found that there was decrease in total –SH groups during frozen storage of all the samples, due to oxidation of SH groups at different pH and which results in the formation disulphide bonds.

Table 4: Changes in total sulfhydryl groups (SH) content of pink perch surimi prepared by conventional method and acid-alkaline solubilization method during frozen storage.

	Sulfhydryl groups content in surimi (µ moles/g protein) during frozen storage (months)				
	0	1	2	3	4
Conventional surimi	6.65±0.031[1]	4.73±0.036[1]	3.66±0.025[1]	3.49±0.031[3]	3.23±0.038[1]
Alkaline surimi	6.16±0.040[2]	3.72±0.020[2]	3.64±0.021[1]	3.72±0.025[1]	2.54±0.032[2]
Acid surimi	6.62±0.015[1]	3.42±0.020[3]	3.43±0.015[2]	3.55±0.031[2]	2.44±0.046[3]

Values are mean±SD. n=3. Values in the same column with different superscripts are significantly different ($p<0.05$)

Changes in Ca^{2+} ATPase activity

As showed in the table 5, the highest Ca^{2+} ATPase activity was found in natural actomyosin (NAM) extracted from conventional surimi followed by Alkaline and acid surimi. During frozen storage, the Ca^{2+} ATPase activity of all the surimi samples decreased while lowest activity was recorded. Ca^{2+}ATPase (EC3.6.4.1) is a myosin heavy chain bond enzyme which carries out the breakdown of ATP into ADP and a free phosphate ion, thereby releasing energy for use of other another biochemical reactions. Myofibrillar protein has an overall net negative charge at the normal pH of surimi. As calcium ions with a divalent positive charge (Ca^{2+}) can form ionic bonds between negatively charged sites of two adjacent proteins. Therefore, addition of Ca^{2+} ions may help to strengthen surimi gel, but only intermolecular ionic bonds

will not induce surimi gelation. Based on this property of calcium as cofactor for endogenous cross-linking enzyme (TGase) in the muscles, the commercial practice of adding calcium salt is improves the gelling property of surimi (Park, 2005) [39]. Roura *et al*. (1990) [40] reported that myofibril ATPase activity has been widely used as a measure of actinomycin integrity. From the result it was found that the Ca^{2+} activity of NAM extracted from conventional surimi was highest as compared to alkaline and acid surimi. Myosin contains 2 identical heavy chains and 2 sets of light chains. As per one of light chains is required for keeping ATPase activities, while the other light chain in globular head regulates the enzyme action (Xiong, 1997) [41]. The present study also supports the observation of alkaline and acid surimi that myosin light chain, containing ATP active sites, is

partially damaged and dissociated during alkaline and acid solubilization process. The measurement of ATPase activities is to estimate the degree of protein denaturation, not a direct assessment of gel forming ability (Choi and Park, 2002) [9]. As the frozen storage time increases the Ca^{2+} activity of NAM extracted from conventional surimi, alkaline and acid surimi were decreases, there was complete loss of ATPase activity in alkaline surimi at the end fourth month and the ATPase activity was lost in acid surimi from second month during frozen storage. This observation may be attributed to the denaturation of myofibrillar protein in the alkaline and acid extraction and also due to frozen storage conditions. This denaturation mainly attributed to partial hydrolysis of peptide

bonds, deamidation of aspargen (Asn) and glutagen (Gln) and instruction of SH groups at alkaline pH which are mostly consider to be irreversible denaturation of proteins (Damodaran, 1996) [10]. The loss of ATPase activity may be due to the changes in tertiary structure caused by ice crystals and the increase in system ionic strength (Benjakul and Bauer, 2000) [42]. Nambudiri and Gopakumar. (1992) [43] found that the ATPase activity of freshwater and brackish water fish was reduced by 70-90% after 6 months of frozen storage at -20°C. Benjakul & Bauer (2000) [42] found that rearrangement of protein via protein–protein interactions was also presumed to contribute to the loss in ATPase activity.

Table 5: Changes in Ca^{2+}-ATPase activity of pink perch surimi prepared by conventional method and acid-alkaline solubilization method during frozen storage.

	Ca^{2+}-ATPase activity changes in surimi (µmole Pi/min/mg) during frozen storage months				
	0	1	2	3	4
Conventional surimi	0.3 ± 0.02^1	0.26 ± 0.02^1	0.22 ± 0.02^1	0.18 ± 0.03^1	0.15 ± 0.04^1
Alkaline surimi	0.10 ± 0.02^2	0.09 ± 0.01^2	0.07 ± 0.01^2	0.03 ± 0.01^2	0
Acid surimi	0.03 ± 0.02^3	0.02 ± 0.01^3	0	0	0

Values are mean±SD. n=3. Values in the same column with different superscripts are significantly different ($p<0.05$)

Gel strength
The gel strength of surimi prepared by three methods i.e conventional, alkaline and acid solubilization deceased with increase in frozen storage time showed in table 6. The initial gel strength of conventional surimi (255.56) was more than alkaline (232.34) and acid (196.34) surimi sample. There was significant decrease in the gel strength as a function of storage time in all the surimi samples. The gel strength was decrease to conventional surimi (227.69), alkaline (206.82) and acid (110.06) surimi at the end of fourth month of frozen storage. The thermally induced gelation is a multi-step process initiated from native myosin sol involving denaturation, protein unfolding, aggregation, and gel network formation as an orderly fashion. During the setting, myosin is denatured and aggregated, and simultaneously polymerized by a calcium dependent endogenous Transglutaminase which is responsible for the setting phenomenon and enhance the surimi gelling properties in a great extent. Proteins that contain both cysteine and cystine groups can undergo polymerization via sulfhydryl-disulphide interchange reactions during heating and form a continuous covalent network upon cooling. Such gels networks that are sustain primarily by non-covalent interaction and are thermally irreversible. This might be attributed to non-removal of protein degrading enzymes like cathepsin B and L with acid treatments. Choi and Park, (2002) [9] recorded similar observation in the case of gel strength of the gels of surimi of pacific whiting (*Meruclccus productus*) prepared using conventional and acid-aided processing and the values of compression force recorded was 110.8 and 92.3 g.cm respectively. Yongsawatdigul and Park (2004) [35] demonstrated that rockfish protein isolates produced from

alkali-aided process had better gel-forming ability as compared to the acid-aided and conventional surimi processes. The muscle proteins being particularly responsible for gelation are myosin and actomyosin (Park *et al*, 1997) [44]. Hultin and Kelleher (2000) [7] reported that acid surimi from Atlantic cod and mackerel produces good gels. Choi and Park (2002) [9] found that the Pacific whiting surimi from 3 washing method made stronger gels than acid -aided process. The lower performance of the isolate from the acid-aided process may be due to the proteolysis found in the low pH solubilization step (Choi and Park, 2002; Undeland *et al*. 2002) [9, 26]. Chaijan *et al*. (2010) [13] also reported that the surimi gel prepared by conventional methods showed greater breaking force and deformation than the alkaline solubilization process. The gel strength of the surimi can be improved by setting the surimi sol below 40°C before cooking (Benjakul *et al*. 2003; An *et al*. 1996; Kimura *et al*. 1991) [45, 46, 47]. The setting phenomenon is attributed to the endogenous transglutaminase (TGase) activity, which can induce protein cross-linking and gel enhancement (Perez-Mateos and Lanier, 2006) [48]. Scott *et al*. (1988) [49] reported that frozen surimi produced by Pollack in Alaska had reduced gel formation when storage time at -29°C increased. MacDonald *et al*. (1992) [50] found that with the increase of frozen storage time, the gel forming ability of hoki muscle decreased with the increase of formaldehyde. The lower gel strength of alkaline and acidic surimi is due to the destruction and dissociation of ATPase sites during the pH-shift processing. During frozen storage, the gel strength of all surimi samples decreased with time, which may be due to protein denaturation and decreased Ca^{2+} ATPase activity.

Table 6: Changes in gel strength of pink perch surimi prepared by conventional method and acid-alkaline solubilization method during frozen storage.

	Gel strength (g.cm) of surimi during frozen storage (months)				
	0	1	2	3	4
Conventional surimi	255.56 ± 2.46^1	239.79 ± 2.85^1	225.23 ± 4.84^1	238.39 ± 4.07^1	227.69 ± 3.22^1
Alkaline surimi	232.34 ± 3.97^2	221.07 ± 3.63^2	220.39 ± 3.19^1	207.99 ± 3.57^2	206.82 ± 6.65^2
Acid surimi	196.34 ± 2.16^3	175.82 ± 2.49^3	164.73 ± 4.09^2	133.16 ± 7.01^3	110.06 ± 2.25^3

Values are mean±SD. n=3. Values in the same column with different superscripts are significantly different ($p<0.05$)

Whiteness

As showed in table 7, the whiteness of surimi gel prepared from conventional surimi, alkaline and acid surimi were recorded as 79.39, 74.51, 58.70 respectively. The higher whiteness value of conventional surimi gel was due to the 3 cycle of washing which removes the dark muscles, as also blood pigments and other water-soluble compounds. The whiteness of alkaline and acid surimi gel was lower than the conventional surimi gel due to the presences of myoglobin in the muscle, skin pigments, haemoglobin etc, which must have solubilized either in alkaline and acid pH processing. Yongsawatdigul and Park (2004) [35] tested the color of acid- and alkali-produced protein isolates and conventionally processed surimi from fish (*Sesbastes flavidus*). They obtained the highest whiteness for conventionally processed surimi followed by acid-produced protein isolate, alkali produced protein isolate, and crude mince. They observed similar values of whiteness for water washed minced, acid and alkali surimi sample as 78.74, 64.84, and 59.18 respectively. Conventional surimi showed the whitest appearance because of the removal of myoglobin during washing. Kristinsson and Liang (2006) [51] tested the color (L*, a*, and b* values) of conventional surimi as well as acid- and alkali-produced protein isolates from Atlantic croaker. They compared the color of isolates and cooked gels. conventional surimi gels had the highest Lightness value when frozen isolates were used, followed by the acid and then alkali-produced protein isolate gels, this was due to the higher level of retained heme proteins after precipitation. Choi and Park, (2002) [9] found lower whiteness of gels from Pacific whiting (*Merluccius productus*) treated with acid and alkali which was due to the solubilisation of hemoprotein in the recovered protein. As the frozen storage time increased the whiteness value of all the surimi samples were found to decrease given in. The decrease in whiteness was also probably due to the adduction of pigment protein, especially oxidised pigment to muscle proteins. In addition, lipid oxidation in muscles during frozen storage may induce cross-linking of pigment proteins and muscle proteins through free radical processes (Saeed *et al*, 1999) [52].

Table 7: Changes in whiteness of pink perch surimi prepared by conventional method and acid-alkaline solubilization method during frozen storage.

		Whiteness values during frozen storage (months)				
		0	1	2	3	4
Convectional surimi	L*	82.27±0.10	77.84±0.86	76.43±1.54	71.12±0.85	70.08±0.62
	a*	0.20±0.05	-0.17±0.25	-0.19±0.17	0.31±0.68	-0.30±0.46
	b*	10.50±0.09	10.33±0.36	10.86±0.32	11.91±1.25	11.40±0.85
	Whiteness	79.39±0.12[1]	75.54±0.66[1]	74.05±1.50[1]	68.74±1.25[2]	67.96±0.41[1]
Alkaline surimi	L*	77.37±0.58	76.86±0.65	73.47±0.83	65.52±3.51	58.68±3.53
	a*	-2.43±0.72	-1.33±0.06	-0.72±0.08	0.28±0.89	-0.16±0.29
	b*	11.46±0.48	12.38±0.15	14.39±0.65	14.11±1.76	10.69±2.68
	Whiteness	74.51±0.54[1]	73.72±0.58[2]	69.80±0.79[1]	62.71±3.19[2]	57.15±2.62[2]
Acid surimi	L*	66.97±0.96	61.12±0.50	59.95±6.12	57.93±1.56	58.39±1.98
	a*	0.17±1.23	0.50±0.20	0.62±0.21	1.37±0.34	3.13±2.40
	b*	24.79±7.56	16.23±1.16	16.46±0.23	17.29±1.93	20±3.66
	Whiteness	58.70±4.01[2]	57.85±0.83[3]	56.59±5.55[2]	54.46±1.63[1]	53.23±3.63[1]

Values are mean±SD. n=3. Values in the same column with different superscripts are significantly different ($p<0.05$)

Expressible moisture content

The gels developed from conventional, alkaline and acid surimi were subjected to estimation of expressible moisture content. Several functional properties of proteins such as solubility, viscosity, water-holding capacity, gelation, emulsification and foaming are depended on water-protein interaction. The ability of protein to exhibit a proper balance of protein-protein and protein-water interactions is critical to their thermal gelation properties. Expressible moisture is amount of water squeezed from protein system by application of the force, by measuring the weight gain of the filter paper surround in the sample. The results showed in table 8, expressible moisture content (%) of conventional surimi, alkaline and acid surimi gel is 2.63%, 6.92%, 7.82%, respectively. Conventional surimi had the lowest expressible moisture content compared with alkaline and acid samples which indicates that the protein network of the gel was higher in water-holding properties during acid/alkali solubilization.

The higher expressible moisture in surimi gel was found in gel samples prepared from alkaline and acid surimi; which was due to the poor gel network of pH-shift process. In addition, adjusting the pH of protein isolate to neutral can enhance the renaturation of proteins to some extent and alter the water-binding property of protein (Rawdkuen *et al*, 2009) [27]. Chaijan *et al*. (2010) [13] reported that higher expressible moisture occurred in the gels of sardine and mackerel muscle prepared by the alkaline process than in those from the conventional washing method. Due to the frozen storage of surimi, there was increased in expressible moisture contents gels as the time increased given in (table 8). This may also be attributed to denaturation of proteins incused by extended frozen storage that has low affinity for water (Benjakul *et al*, 2005) [53]. Therefore, gel matrics that could not imbibe water led to high water release. Higher expressible moisture of surimi gel was closely associated with the poor gel matrix.

Table 8: Changes in gel strength of pink perch surimi prepared by conventional method and acid-alkaline solubilization method during frozen storage.

	Expressible moisture content during frozen storage (months)				
	0	1	2	3	4
Conventional surimi	2.63±0.03[3]	3.97±0.02[3]	4.7±0.03[3]	5.53±0.02[3]	6.33±0.03[3]
Alkaline surimi	6.92±0.03[2]	7.74±0.04[2]	8.55±0.05[2]	9.42±0.03[2]	10.27±0.15[2]
Acid surimi	7.82±0.04[1]	8.41±0.021[1]	9.03±0.06[1]	9.69±0.04[1]	11.13±0.21[1]

Values are mean±SD. n=3. Values in the same column with different superscripts are significantly different ($p<0.05$)

SDS – PAGE

The myosin heavy chain (MHC) band size was found to decreased after the 2nd month of frozen storage of all the samples. Choi and Park (2002) [9] found cathepsin L activity in Pacific whiting muscle proteins treated by the acid solubilization process. And they noted that both acidic and alkaline solubilization processes did not significantly promote proteolysis of MHC as indicated by a minimal loss of MHC in both samples. Just below the myosin bands, approximately 135 kDa, band was seen in alkaline and acid samples and this band was not clearly seen in conventional surimi. These bands were previously reported, and believed to be a result of myosin hydrolysis (Kelleher and Hultin 2000; Kristinsson 2001) [7, 54]. In the present study the pH induced MHC was not observed. Yeung and Jinx-Soo (2005) [55] reported that under acidic conditions, the degradation of MHC in croaker and jack mackerel is higher than that in alkaline conditions.
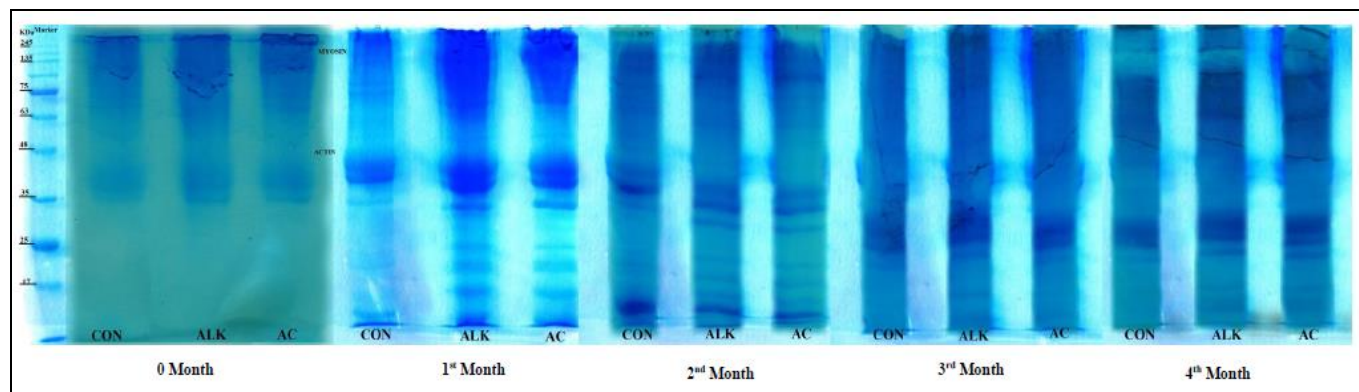

Fig 1: Electrophoretic pattern of pink perch surimi prepared from different washing methods. Lane 1: Marker, CON: Conventional surimi, ALK: Alkaline aided surimi, AC: Acid aided surimi.

Conclusion

This study demonstrated that pH-shift processing was also a successful method for surimi production. The recovery of protein was higher in alkaline-aided process. According to physical and chemical properties, the chemical bonds involved in acid-aided surimi gelation seem to be slightly different from those in conventional surimi. SDS-PAGE shows decreased in myosin heavy chain during frozen storage of all the surimi samples.

Acknowledgement

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