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Protein recovery and gelling properties of striped catfish (*Pangasianodon hypophthalmus*) surimi by different washing methods of surimi processing

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Abstract
In the present study, different washing methods of surimi processing such as conventional, pH-shift processing and alkaline-saline washing process were studied. The protein recovery of surimi extracted by alkaline-aided process (pH-shift) is 69.06% which is higher than other extraction/washing methods. The conventional method gave the highest gel strength value, 403.72 g/cm, followed by the acid-aided method, 374.67 g/cm, the alkaline-aided method, 141.65 g/cm, and the alkaline-saline washing method, 211.83 g/cm. These findings showed a difference in the gel strength of surimi produced using different washing methods that was statistically significant ($p \leq 0.05$). The existence of sarcoplasmic proteins was shown by the presence of a band with a molecular weight of 35 KDa in the alkaline-saline washed, acid-aided, and alkaline-aided surimi samples.

Keywords: Surimi, alkaline-saline washing, pH-shift, gel strength

Introduction
The term "surimi" refers to a wet concentration of myofibrillar protein that has been mechanically deboned, minced, and washed repeatedly in cold water (4-5 °C) and added with cryoprotectants. Washing the fish mince to remove fat and water-soluble proteins is the first stage in a multi-phase process that also includes texturizing and flavoring the mixture to make it taste and look like the desired seafood. Washing process improves the gelling property of surimi and due to this unique property; it has been used in the processing of numerous well-known delicacies, especially kamaboko and chikuwa. The product's quality and the satisfaction of the target market depend largely on the gelation process [14]. Myofibrillar proteins are washed away and swallowed in 2-3% NaCl during the gelation process, after which the remaining protein is separated into myosin and other protein fractions [16, 24]. The hydrophobic and disulfide groups of the amino acids are exposed when the helical structure of surimi denatures during heating. After this, proteins are more likely to aggregate into a three-dimensional network due to the creation of hydrophobic interactions and disulfide bonds [16].
White, low-fat fish like Alaska pollock, Pacific whiting, and threadfin bream are typical ingredients in surimi. Fish with dark flesh and affordable fish have been two examples of new fish resources that have been explored by the surimi business when lean fish stocks have dwindled from overfishing [18]. The raw material from marine resources is not sufficient to meet the needs of the Surimi industry as a result of a decline in marine fish production. Efforts have been made to use inexpensive, underutilized freshwater fish as an alternative source of raw materials for the sustainable manufacturing of surimi.
Exotic and freshwater, the striped catfish (*Pangasius hypophthalmus*) is a member of the family Siluridae. Fresh striped catfish was seldom eaten because of its high fat content and murky scent. The high fat content, off-color, and murky smell of striped catfish make it difficult to process into high-quality surimi. Myofibrillar proteins can be concentrated because the washing process removes undesirable substances like blood, fat, sarcoplasmic proteins, and colours [17, 20].
In India Surimi industry was developed in 1990, and practicing the conventional method for Surimi extraction. Conventional method includes the repeated washing of mince using cold water (5 °C). Since the washing process[13] removes fat and unwanted components (blood, colors, and odorous compounds) and increases the concentration of myofibrillar protein, which improves gel-forming ability, it is important for maintaining surimi quality, but it also results

in low protein/yield recovery due to the loss of most of the water-soluble proteins (sarcoplasmic proteins).

To overcome the technical problems arising while processing surimi from fishes and to improve its quality, Hultin and Kelleher [9] introduced the pH-shift processing, the major aim of this method, alternating acid-based and alkaline-based washing is done due to this, a large amount of sarcoplasmic proteins is recovered and which leads to increase in yield. In conventional method, repeated washing is carried and it requires lot of water and due to repeated washing process most of the water soluble proteins get washed off and this resulted in decrease in protein recovery. Researchers [9] found that the pH-shift approach yielded a greater proportion of protein compared to the standard method. The washing phase is where scientists have found the most significant variation in methodology.

Numerous studies have shown that all of the washing procedures are successful in meeting and even exceeding quality criteria for surimi. However, there is always a need to standardize the number of washing cycles and extraction or washing procedures since results rely on the kind of fish used, its age, size, season, and the place in which it was produced, among other things. In an effort to lessen the environmental impact and save water, the objective of this investigation is to determine whether *Pangasius hypophthalmus* is a suitable raw material for producing surimi in a sustainable way.

Materials and Methods

Fish sample

Freshly captured striped catfish (*Pangasianodon hypophthalmus*) weighing 1-1.5 kg were obtained from a local aquaculture farm in Chiplun, Maharashtra, and transported to the College of Fisheries, Ratnagiri ice boxes in the ratio of 1:1. Fish was manually beheaded and degutted, and bones was removed in a BADDER 600deboning apparatus with a 3 mm perforated drum. Mince was wrapped in a polythene bag and stored in ice boxes filled at 4 °C for further research.

Traditional, pH-shift, and alkaline-saline water washing techniques for surimi preparation

The surimi preparation was carried out by using above raw material by using three different methods i.e., Conventional methods, pH-shift processing and alkaline-saline water washing.

Conventional method

Conventional surimi method was described by [3] a method for preparing conventional surimi. Following a thorough washing in cold water (4 °C) at a water-to-mince ratio of 3:1 (v/w), 10 minutes of gentle stirring, and a filtering through a layer of cheesecloth, the fish mince was dewatered. Three washing steps were performed. A 0.5% Nacl solution was used during the third washing phase, and the ratio of mince to Nacl solution was 1:3.

pH shift process

The acid and alkali solubilization of protein in the pH-shift processing was carried out according to the guidelines given by [9]. Homogenised fish mince and cold distilled water (4°C) at aratio of 1:9 (w/v). Using 2N NaOH for the alkaline and 2N HCl for the acid extraction, the homogenate's pH was adjusted to 11.2 for the alkaline and 2.5 for the acid extraction. After 40 minutes of stirring at room temperature, the homogenate was centrifuged at 6,000 rpm for 20 minutes at 4 °C. Using 2N NaOH or 2N HCl, the alkaline soluble part was collected and adjusted to the isoelectric point of muscle protein (pH 5.5). As soon as the muscle protein was filtered, it was centrifuged at 6000 rpm for 20 minutes at 4 °C to remove the water from the precipitate. At long last, the muscle protein sample had its pH brought up to the standard level at 7.0.

Alkaline-saline washing method

The alkaline-saline washing method of surimi preparation given by [23] was followed. At a 1:4 (w/w) mince/solution ratio, the mince was suspended in a cold (4 °C) alkaline-saline solution (0.15% NaCl in 0.2% NaHCO3). After 15 minutes of gentle mixing, the mince was rinsed and filtered over a maslin cloth. Three times washing was done during the whole process. For the final rinse, we used a cold NaCl solution of 0.5%. In the end, the mince was centrifuged for 10 minutes to separate the surimi.

Protein recovery

To determine which washing technique yielded the most protein recovery from washed mince, we followed the procedure described in [21]. The recovery was determined by weighing the minced fish, which had the same moisture content throughout. The weight of the recovered protein at the same moisture content was determined after surimi was extracted using various washing procedures, and then the moisture level of the washed mince and protein isolates was equalized to 79% moisture (the starting moisture content of fish muscle). The amount of recovered protein was determined using the following formula:

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

Surimi gel preparation

Surimi gel preparation described by [1] has followed to prepare heat-induced surimi gel. To obtain a homogenous solution, partially thawed surimi was mixed with 2.5% salt for 3 mins at 4 °C. It was then filled into a 2.5 cm diameter sized poly-vinylidene casing with both ends tightly sealed. Sols were incubated at 40 °C for 30 mins after being heated in a water bath at 90 °C for 20 mins. The prepared sample was given the name "kamaboko gel" by [4]. Prior to analysis, all gels were placed in cold water to chill for 20 mins before being stored at 4 °C overnight.

Gel strength

At room temperature, the gels were analyzed. The Surimi gels were formed into cylinders and sliced to a length of 2.5 cm. Pieces of each sample were examined for their breaking force (gel strength) and deformation (elasticity/deformability) by being inserted into a texture analyzer with a 5 mm diameter, 60 mm/min spherical plunger. The probe was inserted at a right angle into the gel's slashed surface. The probe was used to penetrate the gel at various depths and the breaking force (g) and distortion (mm) were measured. The breaking force and amount of deformation were used to determine the gel strength of each surimi gel.

SDS-PAGE electrophoresis

The protein band pattern of Surimi produced by different washing method was determined by electrophoresis method given by [12]. Three gram of surimi sample were homogenised

for 1 minute at 11,000 rpm in 27 ml of 5% (w/v) SDS. To dissolve total proteins, the homogenate was incubated for an hour at 85 °C, and then centrifuged to remove the undissolved debris for 20 mins at 3,500 rpm at room temperature. After combining 20 µl of supernatant and 5 µl of sample loading buffer, the tube holding the protein sample was heated in a boiling water bath for three minutes at 100°C. Electrophoresis on a polyacrylamide gel at a constant 110 volts, using a 20 µl protein sample. Coomassie Brillant Blue R-250 (0.02% w/v) was used to stain the protein after separation, and this was followed by destaining using a solution of 50% distilled water, 40% methanol, and 10% acetic acid.

Statistical Analysis

All experiments were repeated three times, and results were summarized as means ± standard deviations. Significant differences between means (p<0.05) were evaluated using analysis of variance (ANOVA). SAS 9.3 was used for the statistical analysis.

Results and Discussions

Recovery/ yield of protein

The protein recovery of surimi extracted from different methods was presented in table no 1. The protein recovery was obtained from conventional method, pH-shift (alkaline-aided and acid-aided) processing and alkaline-saline waster washing were 58.58%, 69.06%, 61.19% and 60.56%. On the whole, it is indicated that the alkaline-aided Surimi (pH-shift) gave better protein recovery followed by acid-aided, alkaline-saline washing method and conventional method. The author [10] observed that in comparison between the alkaline pH (11.0) method yielded 61% to 68% better protein recovery than the acid pH (2.0), which yielded 56% to 61% yield for tilapia (*O. niloticus*). The author [21] found that the acidic pH (85.4%) mince was recovered from tilapia muscle, followed by the alkaline pH (71.5%) and then conventional method (67.9%). The increase in yield at acid and alkaline pH (pH-shift processing) was probably due to recovery of sarcoplasmic proteins. The improved protein recovery in acid/alkali solubilisation can also be attributed to the lowest protein solubility occurring above isoelectric pH of proteins, which is typically lower than acid and alkaline pH values. Considering that most proteins in food are acidic (pH > 7), their solubility is lowest at isoelectric pH (4.0-5.0) and maximum at alkaline pH. The absence of electrostatic repelling force increases aggregation and precipitation through hydrophobic attractions, resulting in the lowest solubility at isoelectric pH. According to [22] the alkaline method yielded a higher yield (61% to 68%) from silver carp (*Hypophtalmichthys molitrix*) than the acid method (56% to 61%), and there was a statistical difference sample (p<0.05). Author [7] observed an inverse association with mince: water ratio and yield in the alkaline-saline procedure. Surimi yield decreases when the mince-to-water ratio increases. The yield for a 1:1 mince: water ratio was 28.41%, and the yield for a 1:2, 1:3, and 1:4 mince: water ratio was 24.90%. Sarcoplasmic protein, fat, blood, and colour have been successfully eliminated with an increased mince: water ratio. The protein recovery/yield obtained is primarily determined by the protein's solubility. The amount of washing cycles, pH of the washing solution, and ionic strength of the washing solution all affect recovery during conventional surimi preparation. The recovery in pH-shift processing is determined by three primary factors: protein solubility at extreme pH acid and alkaline conditions, the size of the sediments created during centrifugations, and protein solubility at the pH selected for precipitation. The acid and alkaline aided procedure recovers the majority of the sarcoplasmic proteins, significantly enhancing yield. In contrast, conventional surimi processing results in the loss of the sarcoplasmic protein throughout repeated washing processes.

Table 1: Protein recovery (%) of surimi extracted by conventional method, alkaline saline washing method and pH-shift processing.

Parameters	Conventional method (CON)	Alkaline-saline washing method (AWS)	pH-shift processing	
			Alkaline-aided surimi (ALK)	Acid-aided surimi (ACS)
Recovery (%)	58.58	60.56	69.06	61.19

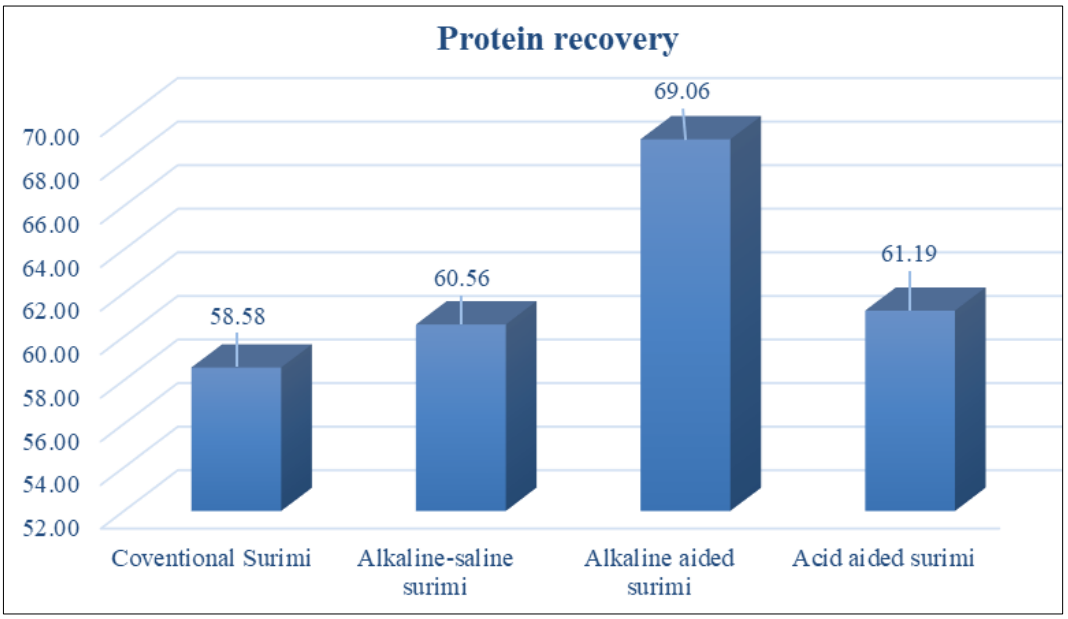

Fig 1: Protein Recovery (%) of conventional surimi, alkaline-saline washed surimi and pH-shift processed surimi (Error bar indicates the SD

Gel strength

The gel strength value of surimi prepared from different extraction methods from striped catfish is presented in the table no 2. The highest gel strength value was recorded in conventional method 403.72 g/cm followed by acid-aided 374.67 g/cm followed by alkaline-aided having gel strength of 141.65 g/cm and lowest was recorded alkaline-saline washing 211.83 g/cm, showed significant difference ($p \leq 0.05$) in gel strength of surimi prepared by different extraction methods (Conventional, pH-shift process, alkaline-saline washing). Denaturtion, protein unfolding, aggregation, and the development of gel network are all steps in the multi-step process of thermally induced gelation, which is started from native myosin sol. A calcium dependent endogenous transglutaminase, which is responsible for the setting phenomena and greatly enhances the surimi gelling characteristics, polymerizes myosin while it is being denatured, aggregated, and polymerized during the setting process. When proteins with both cysteine and cystine groups are heated, sulfhydryl-disulphide interchange reactions can cause polymerization, and when the proteins are cooled, they create a continuous covalent network. Such gels form networks that are thermally irreversible and supported mostly by non-covalent interactions. This may be due to acid treatments failing to remove protein-degrading enzymes like cathepsin B and L. In the present study, the conventional surimi showed the maximum gel strength due to the washing process, which removed the sarcoplasmic proteins simultaneously concentrates the myofibrillar protein that results in increased breaking force in surimi gel explained by [25]. Maximum breaking and deformation force was observed by [21] for kamaboko and modori gel made with conventionally washed surimi followed by an alkaline and acid-aided procedure. For tilapia (*Oreochromis mossambicus*), [19] similarly noted increased gel strength in conventional washed surimi compared to alkaline saline washed surimi. Gel prepared with conventional surimi showed the improved characteristics. During the first two washes, the washing method successfully removed the light chains of tropomyosin, troponin, and myosin. According to author [2] this might obstruct the protein-protein interactions necessary for gel formation. Comparing alkaline saline washing surimi to conventional washing surimi, [1, 6, 18] found the highest gel strength.

It is believed that at their isoelectric pH, proteins are more resistant to denaturation than at any other pH. However, the protein molecules swell and unfold due to significant intra-molecular electrostatic repulsion brought on by high net charge at severe pH values. Extremely alkaline pH values show more unfolding than extremely acidic pH values. The unfolding is due to the ionisation of partially hidden carboxyl, phenolic, and SH groups, which have this impact as they try to expose themselves to the aqueous environment; pH-induced denaturation is largely reversible [5]. When fish proteins are solubilised by acids or alkalis, their conformation and structure are significantly altered, which results in differing properties when the proteins are refolded [11].

Table 2: Gel strength (g/cm) of surimi extracted by conventional method, alkaline-saline washing and pH-shift processing.

Parameter	Conventional method (CON)	Alkaline-saline washing method (ASW)	pH-shift processing	
			Alkaline-aided (ALK)	Acid-aided (ACS)
Gel strength (g/cm)	403.72±6.04[a]	211.83±4.56[c]	141.65±7.36[d]	374.67±4.60 [b]

Values are Mean±SD, n=3.

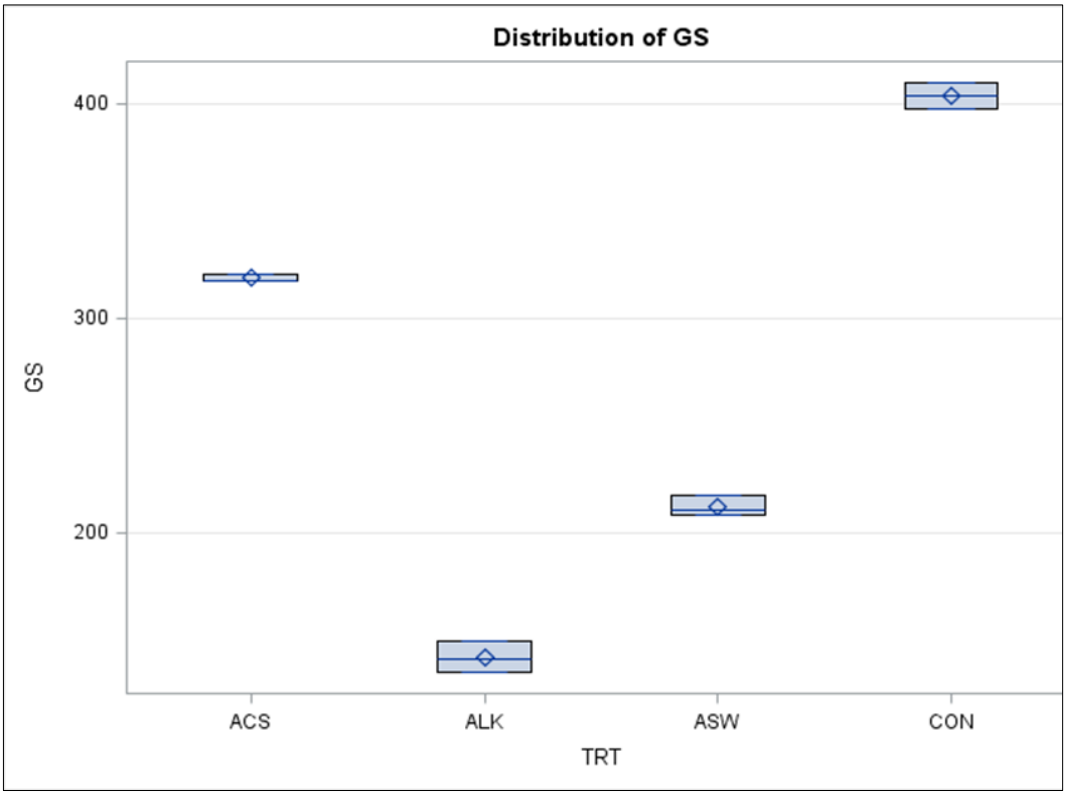

Fig 2: Gel strength (GS) (g/cm) of surimi extracted by conventional method (CON), alkaline saline washing (AWS) and pH-shift processing: acid-aided (ACS) & alkaline-aided (ALK). (Error bar indicates the SD)

SDS-PAGE: Protein pattern of Surimi

The segregated protein bands with respect to molecular weight for striped catfish Surimi prepared by different surimi extraction processing methods were shown in Fig no 3. All surimi samples showed the MHC and Actin in the band with molecular weights of 205 KDa and 45 KDa. Alkaline saline surimi, acid surimi, and alkali surimi all included the band with a molecular weight of 35 Kda, which was indicative of the presence of sarcoplasmic proteins. Author [15] reported the same outcome for samples of tilapia surimi. Additionally, in the present study, alkaline saline surimi, acid surimi, and alkali surimi all had a band with a molecular weight of 35 Kda, indicating the presence of sarcoplasmic proteins. Author [8] reported that the majority of sarcoplasmic proteins had a molecular weight between 30 and 40 KDa. In all processes, actin, troponin, and tropomyosin were shown to be the most frequently obtained proteins, according to [21]. Lowest MHC band intensity was seen in the acid-aided protein isolate.

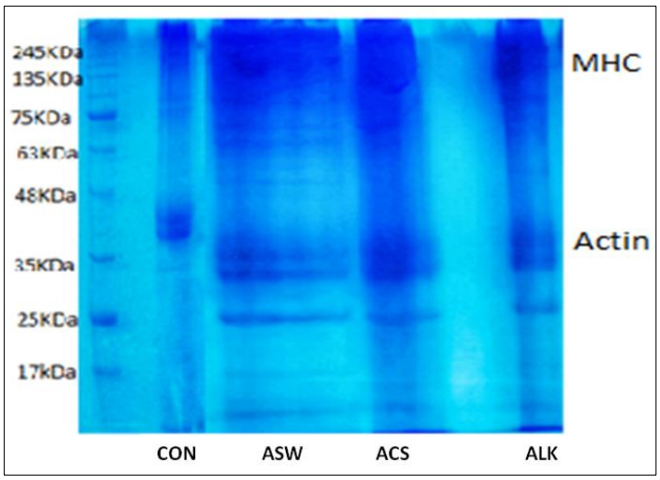

Fig 3: SDS-PAGE, protein pattern of striped catfish surimi prepared conventional (CON), alkaline-saline washing (ASW) method and pH-shift processing (acid (ACS) &alkaline (ALK) solubilization)

Conclusion

In the present study, the protein recovery/yield was recovered highest through pH-shift processing which determines by three primary factors: protein solubility at extreme pH acid and alkaline conditions, the size of the sediments created during centrifugations, and protein solubility at the pH selected for precipitation. The acid and alkaline solubilization recovers the majority of the sarcoplasmic proteins, significantly enhancing the yield.Highest gel strength value was recorded in conventional method because the main components of surimi are myofibrillar proteins, including myosin and actin. Due to their inherent structure and interactions, these proteins can create strong gel networks. When the surimi is heated to the proper temperature leads to protein denaturation and subsequent aggregation, which cause gel formation.

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