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**SD Meshre** College of Fishery Science, Udgir, Maharashtra, India

**DI Pathan** College of Fisheries Science, Shirgoan, Ratnagiri, Maharashtra, India

#### SB Patange

PG Institute of Post Harvest Management, Roha, Raigad, Maharashtra, India

#### MM Shirdhankar

Former Principal Diploma in Fisheries Engineering, Shirgoan, Ratnagiri, Maharashtra, India

AT Markad College of Fishery Science, Udgir, Maharashtra, India

AS Kulkarni College of Fishery Science, Udgir, Maharashtra, India

Corresponding Author: SD Meshre College of Fishery Science, Udgir, Maharashtra, India

### Groundnut husk extract as natural additive for improving functional characteristics of striped (*Pangasianodon hypophthalmus*) catfish surimi

# SD Meshre, DI Pathan, SB Patange, MM Shirdhankar, AT Markad and AS Kulkarni

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#### Abstract

A wet concentration of myofibrillar protein is called Surimi, prepared by deboning and washing of fish muscle. It is a versatile seafood product that is widely consumed worldwide. Protein denaturation and lipid oxidation which affects the gel strength and textural properties is the main challenge to keep the quality and shelf life of surimi. Phenolic compounds, which are naturally occurring bioactive chemicals found in abundance in fruits, vegetables, herbs, and spices, have outstanding antioxidant characteristics that can be used to enhance the physicochemical and sensory characteristics of surimi-based products. By using these natural compounds, surimi-based products can be fortified with bioactive components that may have positive effects on human health, such as antioxidant and anti-inflammatory properties. The use of groundnut husk extract was prepared by using 50% ethanol to improve the textural and functional properties of striped catfish Surimi were studied. This extract was mixed with the surimi at different concentrations i.e. 0% (Control, T<sub>1</sub>), 0.5% (T<sub>2</sub>), 1.0% (T<sub>3</sub>), 1.5% (T<sub>4</sub>) and 2.0% (T<sub>5</sub>). The use of ethanolic (50%) groundnut husk extract T<sub>4</sub> showed highest gel strength values (315.16 g/cm) which were associated with lower expressible moisture content and decreased in TCA-soluble peptides. The SDS-PAGE shows the myosin heavy chain (MHC) and actin in all the concentration but the light bands are observed in 0%, 0.5% and 1%.

Keywords: Surimi, phenolic compounds, functional properties

#### Introduction

Phenolic compounds are a broad group of chemicals that have one or more aromatic rings with at least one hydroxyl group attached. Most plants contain phenolic compounds as secondary metabolites, and they are thought to act as natural antibacterial agents and inhibitors of pre-harvest seed germination O'Connell and Fox (2001)<sup>[7]</sup>. Extracts of phenolic-rich herbs, vegetables, fruits, cereals, nuts, and other plant products are gaining popularity in the food sector as a natural antioxidant ingredient Sang *et al.* (2002)<sup>[17]</sup>. Lipid oxidation is a major cause of quality degradation in muscle foods, particularly in fish which is high in polyunsaturated fatty acids Jittrepotch *et al.* (2006)<sup>[9]</sup>. The relationships between phenolic compounds and proteins play an important role in the processing of certain food items Balange and Benjakul (2009)<sup>[1]</sup>.

Myofibrillar proteins in surimi are mainly involved in the enhancement of gel-forming ability (Benjakul *et al.*, 2003) <sup>[2]</sup>. Various food grade additives, including oxidized phenolic compounds, were utilized to enhance the gel strength of surimi. Groundnut is a major oil and protein producing crop that is consumed raw, pureed, roasted, or blended with other foods in various processed forms. Groundnut meal, groundnut skin, groundnut husk, and groundnut vine are all byproducts of crush peanut processing and harvested groundnut. Groundnut byproducts include a variety of beneficial elements, including protein, fibre, and polyphenolics, which can be added into processed foods to act as functional additives. Groundnut husk extracts containing phenolic compounds may be employed as a natural protein cross-linker. A better knowledge of phenolic compound-protein interactions will be helpful in controlling the functional qualities of proteins in food products as well as the manufacturing of protein components. In addition, the application of phenolic compounds in

an appropriate form and concentration could increase the surimi gel property and other functional properties.

According to fish production statistics, the percentage contribution of freshwater aquaculture is growing internationally. In the Indian freshwater aquaculture environment, apart from Indian major carps and exotic carps, *Pangasinodon hypophthalmus* is a prime important potential species. Pangasius meat is white or light pink in color, and its fillets have no fishy odour, little bones, and have thin skin. These properties, together with its yearround availability in table size, make it an excellent candidate species for the manufacture of surimi and surimibased value-added products to meet consumer demand (Hassan *et al.*, 2017)<sup>[6]</sup>. The aim of the present study was to investigate the effect of ethanolic groundnut husk extract a natural additive to enhance the gelling property of striped catfish Surimi.

#### **Material and Methods**

#### Preparation of groundnut husk powder

The groundnut (*Arachis hypogaea*) was purchased from the Krishi Vigyan Kendra at Shirgoan, Ratnagiri. The groundnut husk was washed and then dried in a solar tent dryer (60 °C) for two days. After that, the husk was pulverised into a fine powder.

#### Preparation of groundnut husk extract

Take 10 g powder in 100 ml of 50% ethanol (1:10 w/v) keep it for 4 hours in a water bath with a shaker at 40 °C. For 30 minutes, the extract was centrifuged at 6000 g. The supernatant was filtered in order to get crude concentrated extract. The groundnut husk extract was then refrigerated in an airtight glass bottle.

### Determination of total phenolic content in groundnut husk extract

Total phenolic content was determined according Folin-Ciocalteu (Jadhav and Anal 2018)<sup>[8]</sup>. Samples (0.4 mL) were combined with 2.0 mL of diluted Folin-Ciocalteu reagent, and the reaction was stopped by adding 1.6 mL of 7.5% sodium carbonate. After 30 minutes at room temperature ( $28\pm1$  °C), the absorbance was measured with a spectrophotometer at 750 nm.

### Preparation of surimi with ethanolic groundnut husk extract

Conventional surimi was prepared according to the method given by Chaijan et al. (2010) [4]. The fish mince was washed in cold water (4 °C) with a water to mince ratio of 3:1 (v/w), the mixture was gently agitated for 10 minutes, and the washed mince was filtered with a layer of cheese cloth and dewatered by squeezing. Washing was preformed three times. Third washing step was carried out with 0.5% NaCl solution, the ratio of mince to NaCl solution was 1:3 (w/w). Cryoprotectants such as 0.2% sodium tripolyphosphate and 4% sucrose were added to wash mince. The surimi was frozen in plastic bag at -40 °C and stored at -20 °C. The ethanolic groundnut husk extract was added to conventional surimi at 0.5%, 1%, 1.5%, and 2% by weight (v/w).

#### Surimi gel preparation

The heat-induced surimi gel was prepared according to method given by Balange and Benjakul (2009)<sup>[1]</sup>.

To obtain a homogenous solution, partially thaw surimi was mixed with 2.5% salt for 3 minutes at 4 °C. It was then fill into poly-vinylidene casing with a diameter of 2.5 cm and both ends of the casing were sealed tightly. Sols were incubated at 40 °C for 30 minutes, followed by heating at 90°C for 20 minutes in a water-bath. This sample was referred to as "kamaboko gel" (Chaijan *et al.*, 2004) <sup>[4]</sup>. All gels were cooled in iced water for 20 minutes and stored overnight at 4 °C prior to analysis.

#### Gel strength

The gels were tested at room temperature. The prepared surimi gels were cut into 2.5 cm diameter cylindrical pieces. The breaking force (gel strength) and deformation (elasticity/deformability) of each sample were tested by placing a piece of each sample into a texture analyzer equipped with a probe with a diameter of 5 mm and a speed of 60 mm/min spherical plunger. The probe was forced perpendicularly into the surface of the Surimi gel until it was punctured. The force in gram (g) required to puncture into the gel (breaking force) and the distance (mm) at which the probe punctured into the gel (deformation), were recorded. Gel strength for each surimi gel was calculated from respective breaking force and deformation.

#### Whiteness

Whiteness of surimi samples was determined by using whiteness meter. L\* (lightness), a\* (redness/greenness), and b\* (yellowness/blueness) were measured in triplicate. Whiteness was calculated using the formula provided by (Shah *et al.*, 2018) <sup>[18]</sup>.

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

#### Expressible moisture content

The method of Ng (1987) <sup>[12]</sup> was used to determine the expressible moisture content. Surimi gel samples were sliced to 0.5 cm thickness, weighed (X), and placed at the top and bottom of the sample between two and three sheets of Whatman paper no. 1 filter paper. At the top, a standard weight (5 kg) was placed and held for 2 minutes. The sample was then removed from the papers and weighed again (Y). The following formula was used to calculate the expressible moisture content:

Expressible moisture content (%) = 
$$\frac{(X - Y)}{X} \times 100$$

#### **SDS-PAGE** gel electrophoresis

The band pattern of protein obtained from different methods was determined by using electrophoresis according to method of Laemmli (1970) <sup>[10]</sup>. Three gram of surimi were homogenised in 27 ml of 5% (w/v) SDS for 1 minute and centrifuged at 11,000 rpm. The homogenate was incubated at 85 °C for 1 hour to dissolve total proteins, followed by centrifugation at 3,500 g for 20 minutes at room temperature to remove undissolved debris. The 20 µl supernatant was mixed with 5 µl sample loading buffer before boiling the tube containing the protein sample at 100 °C for 3 min in a boiling water bath. The sample (20 µl protein) was loaded into polyacrylamide gel subjected to electrophoresis at a constant current of 110 volts. After separation, the protein was stained with 0.02% (w/v) Coomassie Brillant Blue R-

250 and destained using a solution containing 50% distilled water (v/v), 40% (v/v) methanol, and 10% (v/v) acetic acid.

#### **Statistical Analysis**

All analyses were carried out in triplicates and data expressed as means  $\pm$  standard deviations. Analysis of variance (ANOVA) were carried out to assess significant differences between means (p<0.05). All the statistical analysis was carried out using SAS 9.3.

#### **Result and Discussion**

#### Effect of ethanolic extract of groundnut husk

When the 50% ethanolic groundnut husk extract was analyzed for content of phenolic compound against tannic acid as the standard, it was observed that total phenolic content was of GHE was  $157.33\pm5.13$ . The total phenolic compounds extracted from both the husk were observed to be  $157.33\pm5.13$  mg TAE/g. Win *et al.* (2011) <sup>[21]</sup> found that phenolic content of peanut skin (91.74 mg GAE/g). Xu *et al.* (2012) <sup>[22]</sup> found the total phenolic compounds from tea (*Camellia sinensis*) fruit peel (47.50 mg GAE/g dry peel) was obtained under the optimum recovery conditions (43% ethanol, 60 °C, and 33 min). The phenolic content ECHE (ethanolic coconut husk extract) was 453 mg TAE/g ECHE Buamard and Benjakul (2014) <sup>[3]</sup>. Ethanolic kiam wood and cashew wood extracts contains tannic acid at level of 193.0 and 75.0 mg/g dry extract Temdee and Benjakul (2014) <sup>[20]</sup>.

### Effect of ethanolic groundnut husk extract on gel strength

Gel strength of surimi added with different concentration of extract given in Fig 1. There was increasing trend in gel strength with increase in concentration of groundnut husk extract only upto  $T_4$  (1.5% concentration of GHE). The control sample ( $T_1$ ) gel strength was 159.133 g/cm which was increased upto concentration of  $T_4$  (1.5%) is 315.167 g/cm and whereas, the concentration increased the gel strength decreases, the gel strength of control sample ( $T_1$ ) and surimi added with GHE shows significant difference (p < 0.05). The surimi gel sample of bigeye snapper

(Priacanthus tayenus) with 0.05% concentration of oxidized tannic acid and catechin incorporated to surimi had a breaking force value of approximately 500 g; however, as the concentration of these increased from 0.1 to 0.25%, the breaking force value decreased to approximately 300g. Similarly, in the present study, the phenolic content at initial 0.5% raised the gel strength value effectively when adequate oxidation of this molecule occurred. This illustrates the production of quoinon, which was required for protein cross-linking Balange and Benjakul (2009)<sup>[1]</sup>. As a result, the protein gel network developed was sufficiently strong, and the results were consistent with previous studies. Several food-grade substances as well as cross-linking enzymes such as microbial transglutaminases were used Benjakul and Visessanguan (2003)<sup>[2]</sup>. Prigent et al. (2003) <sup>[14]</sup> found that the phenolic compounds can interact with proteins both in non-covalently and covalently. Complexation processes are classified into two types: monodentate and multidentate Haslam (1989)<sup>[5]</sup>. The multidentate mechanism frequently requires a substantially lower phenolic compound/protein molar ratio, and thus less amount of phenolic material. A phenolic compound interacts with only one protein site in the "monodentate" process, thus a larger quantity of phenolic chemical is required (Balange and Benjakul, 2009) <sup>[1]</sup>. Naturally produced plant phenolic compounds have been found to be potential protein crosslinkers (Rawel et al., 2002) [16]. When compared to other phenolic substances such as ferulic acid, catechin, and caffeic acid, oxidized tannic acid (OTA) had the highest gel strengthening effect on bigeye snapper surimi (Balange and Benjakul, 2009)<sup>[1]</sup>. Tannic acid has more hydroxyl groups connected to its aromatic rings, which provides additional protein binding sites (Lopes et al., 1999)<sup>[11]</sup>. In the presence of oxidised tannic acid (OTA), which enhanced protein cross-linking, myofibrillar proteins may aggregate more effectively, resulting in a more compact and dense gel network (Balange and Benjakul, 2009)<sup>[1]</sup>. As a result, while adding phenolic compounds from plant sources can improve surimi gel strength, high concentrations of phenolic compounds exhibited a poorer efficacy with protein.

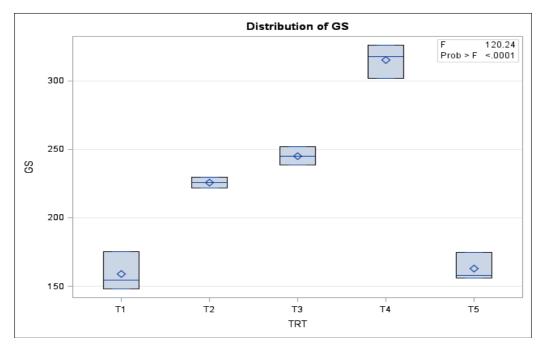


Fig 1: Gel strength surimi treated with groundnut husk extract at different concentration: T<sub>1</sub>: Control, T<sub>2</sub>: GHE 0.5%, T<sub>3</sub>: GHE 1.0%, T<sub>4</sub>: GHE 1.5% T<sub>5</sub>: GHE 2.0% (Error bar indicates the SD)

### Effect of ethanolic groundnut husk extract whiteness of surimi

All gel samples added with ethanolic extract of groundnut husk showed increased in whiteness values with increase in the concentration upto (T<sub>4</sub>). The control sample without addition of groundnut husk (GHE) extract had whiteness value of 61.67. Its value increased to 64.61, 65.40 and 66.86 with addition of 0.5, 1, 1.5% concentration of groundnut husk extract respectively. When oxidized phenolic compounds at a level of 2% were added, there is decreased in whiteness of surimi which shows significant difference (p<0.05). Whiteness values of surimi with addition of extract of groundnut husk at different concentrations were found to be 61.67, 64.61, 65.40, 66.86, and 64.44 in the present study. Whiteness values reduced with increasing extract concentration observed in 2.0% (T<sub>5</sub>). The addition of oxidised phenolic substances reduced the whiteness of bigeye snapper surimi gel (Balange & Benjakul, 2009)<sup>[1]</sup>. The addition of relatively small amounts of phenolic compounds may have little impact on the colour of the resulting gel from dark meat fish surimi, which is normally dark in colour. However, many food grade additives were used to improve the gel strength of surimi, and the addition of these ingredients resulted in undesirable effects on the surimi gel, mainly off flavour or off colour (Rawdkuen & Benjakul, 2008) <sup>[15]</sup>. Because of their impact on flavour and colour, phenolic compounds have been recognized as critical in plant food, particularly enzymatic browning, which could have resulted in a fall in whiteness values with an increase in phenolic content (Rawdkuen and Benjakul, 2008) <sup>[15]</sup>. In the present study, the addition of low concentrations of phenolic compounds had no negative influence on the colour of the resulting gel form from striped catfish surimi.

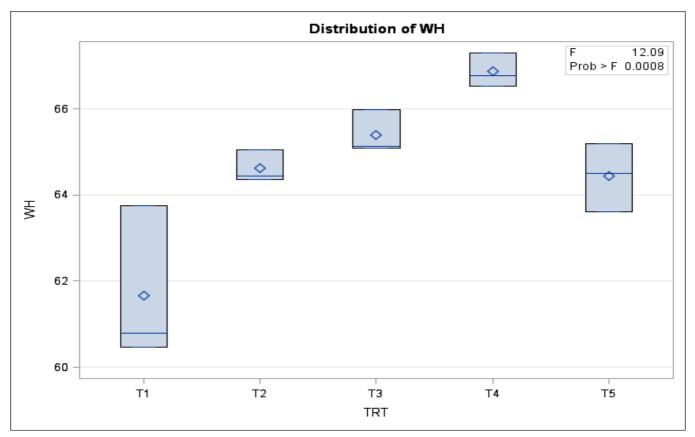
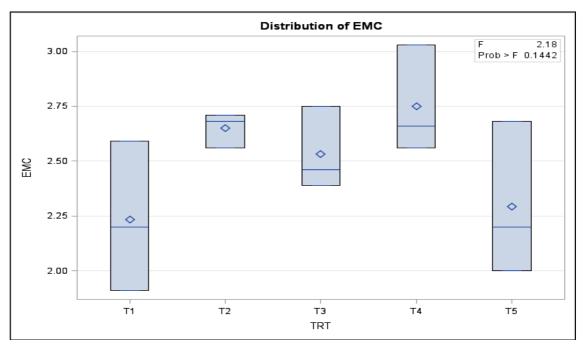


Fig 2: Whiteness values of surimi treated with groundnut husk extract at different concentration: T<sub>1</sub>: Control, T<sub>2</sub>: GHE 0.5%, T<sub>3</sub>: GHE 1.0%, T<sub>4</sub>: GHE 1.5%T<sub>5</sub>: GHE 2.0% (Error bar indicates the SD)

## Effect of ethanolic groundnut husk extract on expressible moisture content

The expressible moisture content of surimi gel samples was similarly influenced by the addition of ethanolic groundnut husk extract. When ethanolic GHE extracts were added to surimi at various concentrations, the expressible moisture content of the surimi gels increased (p < 0.05) as compared to the control. It was 2.23% in surimi gel samples without GHE extract, which increased to 2.75% when ethanolic groundnut extract was added at the concentration of (T<sub>5</sub>) 2%. Balange and Benjakul (2009) <sup>[1]</sup> found 3.93 and 17.25% expressible moisture content in untreated bigeye snapper and mackerel surimi. The value was reduced to 2.55% with 0.05% oxidized tannic acid treated surimi and 3.26% with

0.5% oxidized tannic acid treated surimi, respectively. This proved that the water-holding capacity of surimi gels might be increased by adding phenolic compounds at suitable quantities (Balange and Benjakul, 2009) <sup>[1]</sup>. Proteins were denaturated and eventually aligned themselves to form the network, which can absorb water, throughout the gel mechanism process of setting at 40 °C (Benjakul & Visessanguan, 2003) <sup>[2]</sup>. The inclusion of phenolic compounds could improve protein cross-linking, which leads to the creation of strong links with enhanced water-holding capacity. In general, the water retaining capacity of gel with phenolic sused. The fall in gel strength correlated with the decrease in water holding capacity.



**Fig 3:** Expressible moisture content (%) of surimi treated with groundnut husk extract at different concentration: T<sub>1</sub>: Control, T<sub>2</sub>: GHE 0.5%, T<sub>3</sub>: GHE 1.0%, T<sub>4</sub>: GHE 1.5% T<sub>5</sub>: GHE 2.0% (Error bar indicates the SD)

### Effect of ethanolic groundnut husk extract on protein solubility

The groundnut husk extract also affected the protein solubility of surimi. GHE extracts when added to surimi at different concentrations the protein solubility increased (p<0.05) as compared to that of control (T<sub>1</sub>) 66.52% whereas, it shows decreased in T<sub>3</sub> (72.30%) sample and highest protein solubility was observed in T<sub>2</sub> (85.65%) The solubility of surimi treated with groundnut husk extract shows the increasing trend. It may be due to the native myofibrillar proteins which are normally soluble in high ionic strength buffer. The decrease in solubility reflects the development of protein masses during the gelation process. During heating, proteins underwent denaturation and aggregation to form a three-dimensional structure (Stone &

Stanley, 1992) <sup>[19]</sup>. Increases in solubility were observed in T<sub>2</sub>, which indicates the presence of hydrophobic and hydrogen bonds in surimi. The hydroxyl groups of phenolic compounds may interact with the nitrogen or oxygen of arginine, asparagine, lysine, histidine, glutamine aspartic acid, glutamic acid, serine, threonine, tyrosine, cysteine, and tryptophan as a hydrogen acceptor, and quinone protonation may occur to some extent following neutralisation (Prigent, 2005) <sup>[13]</sup>. As a result, hydroxyl groups may be partially regenerated and interacted with proteins via hydrogen bonding. Prigent (2005) <sup>[13]</sup> describes hydrophobic interactions between phenolic chemicals and hydrophobic amino acids such as alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, tryptophan, cysteine, and glycine residues.

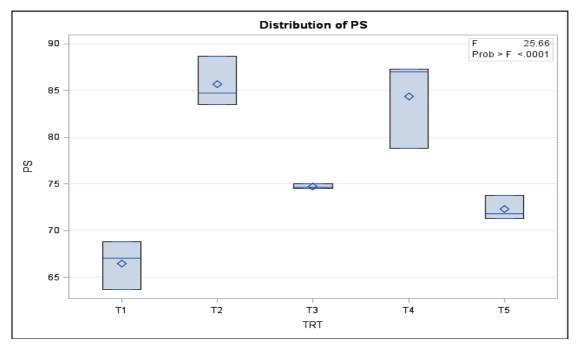
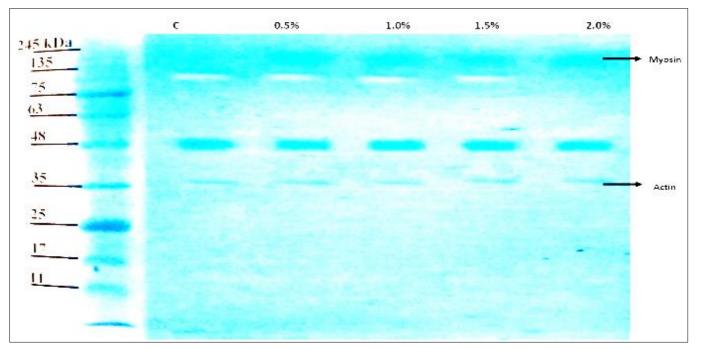


Fig 4: Protein solubility (%) of surimi treated with groundnut husk extract at different concentration: T<sub>1</sub>: Control, T<sub>2</sub>: GHE 0.5%, T<sub>3</sub>: GHE 1.0%, T<sub>4</sub>: GHE 1.5% T<sub>5</sub>: GHE 2.0% (Error bar indicates the SD)

#### Effect of ethanolic groundnut husk extract on SDS-PAGE protein pattern

SDS-PAGE of GHE and CHE treated surimi for different concentration is shown in fig 5. it can be seen that intensity of protein band pattern did not differ much between GHE and CHE and different concentration of phenolic compounds. Myosin light chain between 245 to 180 kDa was observed, and actin protein band was also observed in all surimi samples. Actin was more prominent protein in gel at 45 kDa. The SDS-PAGE protein patterns of surimi with addition of ethanolic groundnut husk and coconut husk extract at various concentrations are shown in fig 5. The dominant proteins in the surimi paste were myosin heavy chain bands and actin. Untreated samples showed a decrease in myosin heavy chain bands as compared to treated samples, which could be attributed to the formation of crosslinking via SS bonds, which fixed the myosin heavy chain

during setting. As with mackerel Surimi, Benjakul and Visessanguan (2003)<sup>[2]</sup> observed a decrease in MHC of surimi gel from bigeye snapper when the setting was used. The intensity of the actin band was quite noticeable in both the control and treated surimi samples. Because of variations in protein patterns between gels with different concentrations of phenolic compounds, the protein molecules may be cross-linked differently depending on the level of phenolic compounds present. Covalent alteration of proteins by oxidation of alkaline pH products has been extensively described. It was proposed that oxidized phenolic compounds could reduce the proteolysis produced by endogenous proteinase because cross-linked proteins are less vulnerable to proteolysis (Rawel, 2002)<sup>[16]</sup>. This could be related to gel strengthening as well as increases in protein cross-linking.



**Fig 5:** SDS-PAGE of surimi with ethanolic grountnut husk extract at different concentration 1<sup>st</sup> lane: protein ladder, 2<sup>nd</sup> lane: control, 3<sup>rd</sup>-6<sup>th</sup> lane: different concentration of extract of ethanolic grountnut husk extract

#### Conclusion

Surimi added with 1.5% concentration of ethanolic groundnut husk (EGH) extract showed increase in the gel strength of surimi gel samples by 315.167 g/cm as compared to control sample. The whiteness reduced as the extract concentration increased. The SDS-PAGE analysis identified myosin heavy chain (MHC) and actin at all concentrations, although the lighter bands were visible at higher concentrations. Surimi gel can be strengthened by hydrogen bonds and other interactions because phenolic components are high in hydroxyl groups. Striped (*Pangasianodon hypophthalmus*) catfish surimi gel strength can be improved by using appropriate quantities of ethanolic groundnut husk extract.

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