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Effect of groundnut husk extract on functional characteristics of pink perch (*Nemipterus japonicus*) surimi

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Abstract
Groundnut skin and husk are by-products of groundnut processing industry. These are considered as agro-waste and often used in animal feedstuffs and fertilizers. Husk is a good source of phenolic compounds that have antioxidant and antibacterial properties. The interactions between phenolic compounds and proteins also play a role in the processing and quality enhancement of certain food products. The present study aims to investigate the effects of ethanolic groundnut husk extract on gel enhancement of pink perch (*Nemipterus japonicus*) surimi. The extraction of phenolic compounds was done in 50% ethanol and the extracted material was added to surimi at concentrations of 0.5, 1, 1.5 and 2 % on w/w basis. The dried husk had total phenolic content of 20.55 mg TAE/g powder. The surimi had highest gel strength with 0.5% and 1% concentration of extract. The whiteness and expressible moisture content of surimi decreased as the concentration of extract was increased. The SDS-PAGE showed myosin heavy chain (MHC) and actin in all the concentrations but the lighter bands were seen with higher concentrations. Phenolic compounds are rich in hydroxyl groups, surimi gel can be strengthened via hydrogen bond and other interactions. Ground nut husk extracts at an appropriate level could improve gel strength of pink perch Surimi.

Keywords: Groundnut husk extract, phenolic compounds, surimi gel

1. Introduction
A phenolic compound is a group of chemical substances having one or more aromatic rings to which at least one hydroxyl group is attached. Most plants produce phenolic compounds as secondary metabolites and may act as natural antimicrobial agents and inhibitors of pre-harvest seed germination [9]. Extracts of herbs, vegetables, fruits, grains, nuts and other plant products are rich in phenolic substances, as natural antioxidants; they are increasingly interested in the food industry [12].
Myofibrillar proteins in surimi are mainly involved in the enhancement of gel-forming ability [4]. To increase the gel strength of surimi, various food grade ingredients including oxidised phenolic compounds have been used. Groundnut is one of the most important oil and protein producing crop in the world which is consumed as raw, pureed, roasted or mixed with other foods in different processed form. Several groundnut by-products are produced from crush peanut processes and harvested groundnut, including groundnut meal, groundnut skin, and groundnut husk and groundnut vine. The by-products of groundnut contain many functional compounds, such as protein, fiber and polyphenolics, which can be incorporated into processed foods to serve as functional ingredients. Extracts from groundnut husk containing phenolic compounds could be used as natural protein cross-linker. The present study shows the effect of groundnut husk extract on functional characteristics of pink perch (*Nemipterus japonicus)* surimi.

2. Material and methods
2.1 Preparation of groundnut husk powder
Groundnut (*Arachis hypogaea*) was purchased from the krishi vigyan Kendra, Shirgoan, Ratnagiri. The groundnut shell (husk) was hand-shelled, and it was washed and dried in solar tent drier (60 ^{0}C) for 2 days. Then ground the husk into fine powder.

2.2 Preparation of groundnut husk extract

Take 10 g powder and was dissolved in 100 ml of 50% ethanol (1:10 w/v), and prepared Extract was evaporated at 40°C for 4 hours in a water bath. The extract was centrifuged at 6000*g* for 30 min. Supernatant was filtered to obtained a concentrated crude extract. The ethanolic groundnut husk extracts were stored at 4 ^{0}C until use.

2.3 Determination of total phenolic content in groundnut husk extract

The quantification of total phenolic compounds in groundnut husk extract was carried out according to the Folin-Ciocalteu procedure as described by [6]. Samples (0.4 mL) were mixed with 2.0 mL of the Folin- Ciocalteu reagent (diluted 10 times), and the reaction was terminated using 1.6 mL of 7.5% sodium carbonate. After 30 min incubation at room temperature (28±1°C), the absorbance was read at 750 nm using a spectrophotometer. The extract was measured in triplicates against tannic acid standard curve and the results were expressed as mg TAE/g of dry sample.

2.4 Preparation of surimi with groundnut husk extract

The ethanolic groundnut husk extract were added to conventional surimi of pink perch @ 0.5%, 1%, 1.5% and 2% on weight basis (v/w).

2.5 Surimi gel preparation

The heat-induced surimi gel was prepared according to [7] prepare the gel, frozen mince, was thaw for 30 min in running water (26-28 ^{0}C) until the core temperature reached 0 ^{0}C, the samples were cut into small pieces (1 cm thickness) and moisture content was adjusted to 80 %. Dry Sodium chloride was added to the sample (2.5%, w/w) and mince was chopped for 4 min at 40 ^{0}C to obtain homogeneous sol. The sol was then stuffed into polyvinyl dine casing with the diameter of 2.5 cm and both ends of casing were sealed tightly. The sol was then incubated at 40 for 30 min, followed by heating at 90 ^{0}C for 20 min in a water bath. The gels were cooled in iced water and stored for 24 hours at 40 ^{0}C prior to analysis.

2.6 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.

2.7 Whiteness measurement of surimi gel

Whiteness of surimi gels was measured using a Hunter lab Miniscan EZ Model No. 4500L described by [13]. The measurement of L*(lightness), a* (redness/green) and b* (yellowness/blueness) was performed in five replications. Whiteness was calculated using the following equation.

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

2.8 Expressible Moisture of surimi gel

Expressible moisture content of surimi gel samples was measured according to the method of [3] with a slight modification. Gel samples were cut into a thickness of 5 mm, weighed (X) and placed between 3 pieces of what man paper No. 4 at the bottom and 2 pieces on the top of the sample. The standard weight (5 kg) was placed at the top and held for 2 min. The samples were then removed from the papers and weighed again (Y).

Expressible moisture content was calculated using the following equation:

$$\text{Expressible moisture content (\%)} = 100[(X-Y)/X]$$

2.9 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 [8] using 5% stacking gel and 8% separating gel. Sample (3g) was homogenized with 27 ml of 5% (w/v) SDS for 1 minute at a speed of 11,000 rpm. The homogenate was incubated at 850 C for 1 hr to dissolve total proteins, followed by centrifugation at 3500g for 20 minutes at room temperature, to remove undissolved debris. The 20 µl supernatant were mixed with 5 µl sample loading buffer and boiled the tubes containing protein sample at 100 ^{0}C for 3 minutes in a boiling water bath. The sample (20 µl protein) was loaded into polyacrylamide gel and subjected to electrophoresis at a constant current of 110 volts. After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 and distained with solution containing 50% distilled water (v/v) and 40% (v/v) methanol and 10% (v/v) acetic acid.

2.10 Statistical analysis

All analyses were carried out in triplicates and data expressed as means ± standard deviations. Analysis of variance (ANOVA) were carried out to assess significant differences between means ($p<0.05$).

3. Results and discussion

In the present study, the phenolic content in groundnut husk extract was analysed against the tannic acid and estimated phenolic content was observed to be 20.55 mg TAE/g. [15] found that phenolic content of peanut skin (91.74 mg GAE/g). Phenolic compounds (e.g., caffeic acid) can interact with proteins, which results in the improvement of the network formation in gelatin gels, rendering improved mechanical properties and higher thermal stability [14]. To increase the gel strength of surimi several food grade ingredients have been tried but the addition of these may pose adverse effects on the surimi gel particularly off-flavour or off-colour [10]. Phenolic content in 60%, 80% 40% and 100% ethanol extract from coconut husk as 464, 454, 488 and 256 ml TAE/g reported by [4] sample respectively. They observed that a phenolic content was higher when ethanol concentration increased upto 60%. Hence, an alternative food grade in as phenolic compounds as groundnut husk was tried to increase the gel strength of surimi because naturally derived plant phenolic compounds have been showed as the protein cross-linkers [11]. Addition of these compounds has been shown to increase the gel property of mackerel surimi [1].

3.1 Effect of ethanolic groundnut husk extract on functional characteristics of pink perch surimi.

The gel strength of surimi was increased as the concentration of extracts increased (Fig 1). Surimi added with 0.5% and 1% concentration of ethanolic groundnut husk (EGH) extract showed increase in the gel strength of surimi gel samples by 22.33% and 20.82% respectively as compared to control sample. As the concentration of phenolic compounds increased further, the gel strength of surimi decreased,

wherein with 1.5% and 2% concentration levels of EGH showed decrease in the gel strength as compared to 0.5 and 1.0% levels and the gel strength of control sample and surimi with addition of ethanolic groundnut husk extract has significant difference ($p<0.05$). A breaking force value of approximately 500g has been observed for the surimi gel sample of bigeye snapper (*Priacanthus tayenus*) with 0.05% concentration of oxidized tannic acid and catechin incorporated to surimi however with further increase in the concentration of these from 0.1 to 0.25% there was significant drops in breaking force value to about 300g. In the present study the phenolic content at initial 0.5% increased the gel strength value effectively after adequate oxidation of this compound taken place. This reveals the formation of quoin on which was necessary for the cross-linking of proteins [1]. As a result the protein gel network obtain was strong enough and results were in agreement with the previous work.

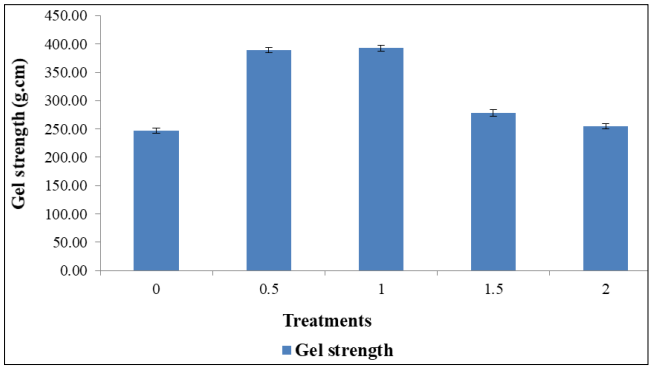

Fig 1: Effect of ethanolic groundnut husk extract on gel strength of surimi gel of pink perch surimi. (Error bars indicate the SD)

As observed in the present work whiteness values of surimi with addition of ethanolic extract of groundnut husk at different level were 79.56, 77.46, 77.13, 73.16 and 71.45 respectively (Fig 2). The whiteness was decreased with increased in extract concentration. Addition of oxidised phenolic compounds resulted in the decrease in whiteness of gel of bigeye snapper surimi [1]. Addition of phenolic compounds at very low amounts might have no negative effect on the color of the resulting gel from dark flesh fish surimi, which is generally dark in color. To increase the gel strength of surimi, various food grade ingredients have been used but the addition of these ingredients poses adverse effects on the surimi gel, particularly off flavour or off colour [10].

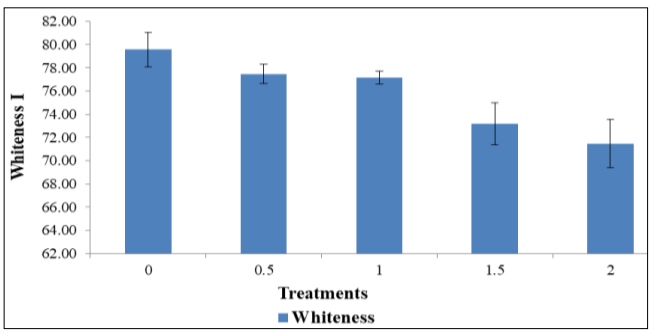

Fig 2: Effect of ethanolic groundnut husk extract on whiteness of surimi gel of pink perch surimi. (Error bars indicate the SD)

The addition of ethanolic groundnut husk extract affected the Expressible moisture content of surimi gel samples given in Fig 3. The expressible moisture content of untreated surimi samples was 4.23%. When EGH extracts added at different concentrations the expressible moisture content of pink perch surimi gels decreased ($p<0.05$), as compared to that of control. Observed values 3.93 and 17.25% of expressible moisture content in untreated bigeye snapper and mackerel surimi respectively [1]. The value of which was reduce to 2.55% with 0.05% oxidized tannic acid treated surimi and 3.26% with 0.5% oxidized tannic acid treated surimi respectively. The observations made in the present work were found to be in agreement with the previous researchers. This indicated that the water-holding capacity of surimi gels could be improved with the addition of phenolic compounds at optimal levels [2]. In the gel mechanism process of setting at 40 ^{0}C, proteins underwent some denaturation and aligned themselves gradually to form the network, which can imbibe water [4]. With addition of phenolic compounds the cross-linking of proteins could enhance, which resulted in formation of strong network with greater water-holding capacity.

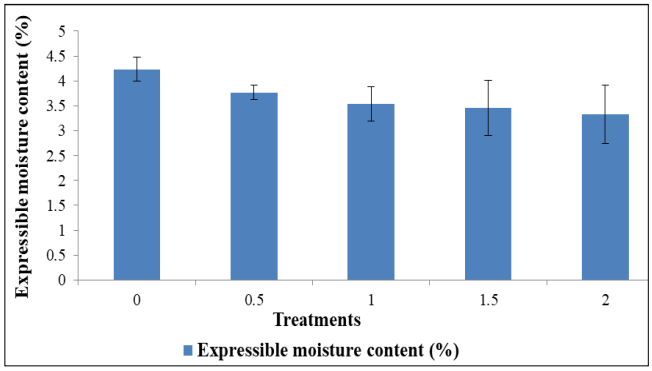

Fig 3: Effect of ethanolic groundnut husk extract on expressible moisture content of surimi gel of pink perch surimi. (Error bars indicate the SD)

The SDS –PAGE protein patterns of surimi with addition of ethanolic groundnut husk extract at different concentrations is given in fig 4 the surimi paste contained myosin heavy chain bands and actin as the major proteins. Decrease in myosin heavy chain bands in untreated has been observed when compared with treated samples which could be due to formation of cross-linking by SS bonds that stabilized the myosin heavy chain during setting. As observed in mackerel surimi [4] reported decrease MHC of surimi gel from big eye snapper particularly when the setting was implemented.

The intensity of actin band was quite prominent in the control as well as treated surimi samples. Due to the difference in the protein patterns amongst gels with addition of different concentration of phenolic compounds, the protein molecules might be cross-linked differently with level of phenolic compounds. Covalent modification of proteins by the oxidation of products generated at alkaline pH was reported extensively. It was suggested that oxidized phenolic compounds might partially lower the proteolysis caused by endogenous proteinases since, cross-linked proteins are likely less susceptible to proteolysis [11]. This might be associated with the gel streng thing in addition to enhance in protein cross-linking.

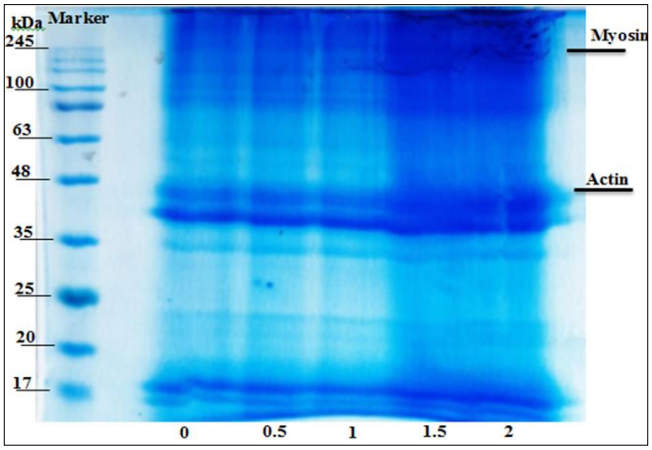

Fig 4: SDS – PAGE of pink perch surimi treated with ethanolic groundnut husk extract. Marker, Lanes: 0, 0.5, 1, 1.5, 2% EGH extract.

4. Conclusion

Surimi added with 0.5% and 1% concentration of ethanolic groundnut husk (EGH) extract showed increase in the gel strength of surimi gel samples by 22.33% and 20.82% respectively as compared to control sample. The whiteness was decreased with increased in extract concentration. The SDS-PAGE showed myosin heavy chain (MHC) and actin in all the concentrations but the lighter bands were seen with higher concentrations. Phenolic compounds are rich in hydroxyl groups, surimi gel can be strengthened via hydrogen bond and other interactions. Appropriate concentrations of ethanolic groundnut husk extract can improve the gel strength of pink perch surimi.

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