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Improvement in Textural Properties of Mackerel (*Rastrelliger kanagurta*) Surimi by Using Seaweed (*Sargassum tenerrimum)* Extract as Natural Gel Enhancer

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ABSTRACT

Surimi is a Japanese term for washed fish mince which can be used as a base material for manufacturing different analogue products like shrimp, lobster, crab analogues etc. Low cost and lean fish is generally used for making surimi but its over exploitation resulted in its stock depletion recently. Dark muscle fish like mackerel can be an alternative raw material but it has low gel forming ability. An attempt has been made in the present investigation to extract phenolic compounds from seaweed (*Sargassum tenerrimum)* and use as cross-linker in mackerel (*Rastrelliger kanagurta*) surimi to enhance its gel forming ability. Seaweed extract contained total phenolic compounds of 16.24 ± 0.32 mg tannin/ gm of dry seaweed powder, added at different levels (0.5 – 2.5% of total weight of surimi) in mackerel surimi to check the effect on the properties of gels from mackerel surimi. Surimi added with 2.0% seaweed extract had the increases in gel strength by 32.45% and also lowered expressible moisture content. Decreases in whiteness ($P < 0.05$) and increase in pH ($P > 0.05$) were observed with increasing seaweed extract concentration, compared with control sample. SDS-PAGE data revealed the slight disappearance of myosin heavy chain with the incorporation of 1.5 and 2% seaweed extract in mackerel surimi gel. Thus, the seaweed extract @ 1.5 or 2% can be used as surimi gel enhancer for mackerel without affecting its sensory properties.

Key words *: Cross-linking, Gelation, Seaweed, Mackerel, Surimi.*

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INTRODUCTION

Surimi gel is a three-dimensional myofibrillar protein network. The textural properties developed during gelation are normally expressed in terms of gel strength, which is the basic parameter for determining the quality and price of surimi (Benjakul *et al.* 2004a). It can be affected by both intrinsic and extrinsic factors including species, freshness, endogenous enzymes, additives as well as cooking procedure (Benjakul *et* . 2004a). In general, the lean fish have been used for the surimi production. Due to overexploitation, the availability of such fish is reducing. The use of small pelagic fish species, such as mackerel could be a better alternative for the lean fish but their use for surimi production is limited mainly due to the large quantity of lipids and myoglobin in the muscle tissue (Chaijan *et al.* 2004). Furthermore, pelagic fish has been found to possess the high proteolytic activity, which is associated with gel softening. To alleviate the problem, protein additives have been widely used to enhance the gel strength of the surimi via inhibition of proteolysis caused by an endogenous proteinase (Benjakul *et al*. 2004b). There have been a few studies describing the cross linking ability of phenolic compounds with proteins (Rawel *et al*. 2002; Strauss and Gibson 2004). Balange and Benjakul (2009a) reported a significant increase in the gel strength of big eye snapper surimi when commercial phenolic compounds in oxidised forms were added. Among all oxidised phenolic compounds used, oxidized tannic acid (OTA) exhibited the highest gel strengthening effect, compared with oxidized ferulic acid, oxidized catechin and oxidized caffeic acid (Balange and Benjakul 2009a).

Brown seaweed (*Sargassum tenerrimum*) is very common in the west coast of India. Seaweed contains phenol level up to 20 % of their dry weight (Connan and Stengel 2007). Tannin substances with phenolic character occur in marine algae in the physodes of Phaeophyta, such as *Sargassum species* (Vimalabai *et al*. 2004). Seaweeds have been identified as a rich source of bioactive compounds and also exhibiting antimicrobial potential against the pathogenic microbes of medical, agricultural and environmental importance. The bioactive molecules-phenolic compounds i.e. the secondary metabolites, mainly as a phlorotannins are found at high level in marine brown algae (Ragan and Glombitza 1986). The preparation of seaweed extract containing phenolic compounds could increase the value of those seaweeds and the novel natural additives can be applied in food industry, especially surimi industry. Recently, Shitole *et al*. (2014) had also reported the effective cross-linking of phenolic compounds extracted from seaweed towards myofibrillar proteins of lesser sardine.

However, there is a little information on the utilization of seaweed extract as the cross-linking agents in food proteins, particularly mackerel myofibrillar proteins. Therefore, in the

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present study an attempt was made to extract and quantify tannin in the seaweed (*Sargassum tenerrimum*) and to use the extracts as gel strengthener in surimi from mackerel (*Rastrelliger kanagurta*).

MATERIALS AND METHODS

Chemicals: Tannic acid Powder Pure (Molychem, Product code: 19280), sodium carbonate (Thomas Dekker, Mumbai), Folin-Ciocalteu Phenol reagent (Sisco Research Laboratory) were obtained.

Collection of Brown Seaweed: Brown seaweed (*Sargassum tenerrimum*) species available along Ratnagiri coast were collected by hand picking method. Collected samples were packed in polyethylene bag and transported to the Department of Fish Processing Technology, College of Fisheries, Ratnagiri within 2 h. Collected seaweeds washed with fresh water and kept for sun drying for 14 days (Shitole *et al*. 2014).

Extraction of Phenolic Compounds from Dry Seaweed: The sundried seaweeds were ground using a portable grinding machine (Kenstar, Senator, Japan). Then grinded seaweed powder was sieved with Test sieve (Jayant scientific IND, India) using a screen with a diameter of 0.07 mm. Seaweed extract prepared according to the method described by Zahra *et al*. (2007) with slight modifications.

Seaweed powder of 1gm was dissolved in 100 ml distilled water and autoclaved at 121^0C for 15 min (Shitole *et al*. 2014). The autoclaved treatment followed by centrifugation (5000 rpm for 10 min), with centrifuge (Hettich Zentrifugen, D-78532; Germany) and then the supernatant was evaporated directly on flame and extract was collected in glass bottles.

Quantification of Total Phenolic Content: Quantification of total phenolic compounds in seaweed extract was carried out according to the method of Kuda *et al*. (2005). Briefly, 0.4 ml aliquot of the seaweed extract from dilution of sample was transferred into a test tube containing 0.8 ml of the 10% Folin-Ciocalteu- phenol reagent. After 3 min, 1.6 ml of the 10% sodium carbonate solution was added. The contents were mixed, using glass rod and left to stand at room temperature for 1 h in dark. Absorbance was recorded at 750 nm using a spectrophotometer.

Tannic acid pure was used as standard for quantification of phenolic content in seaweed. Absorbance for tannic acid solution was recorded at different concentrations. Recorded absorbance of tannic acid was used for quantification of phenolic content in seaweed, with the help of absorbance recorded from seaweed extract. Estimation of the Phenolic contents was carried out in triplicates and the results were expressed as mg of (+) Tannic acid equivalent (TA)/gm dry sample. Blank for each extract was prepared in the same manner, except that distilled water was used instead of sample. From our previous study, we found that 1 gm powder of dried seaweed yielded 16.24 mg of total phenolic content (Shitole *et al*. 2014).

Surimi Gel Preparation: Indian mackerel surimi was purchased from M. D. Naik, surimi plant, Ratnagiri. Surimi was prepared by using 1 part of mackerel mince to 3 part of chilled water (1:3 ratio of mince: chilled water) with three washing cycles. This washed surimi was then passed through rotary screen and then to refiner unit for removing fine skin, bone or scale parts. Finally surimi was passed through the screw press to remove excess water. Proximate composition of surimi: Moisture- 77.5%, Protein-18%, Fat-1.0% and Ash- 1.0%. Surimi gel was prepared according to the method described by Balange and Benjakul (2009b) with slight modification. Frozen surimi was tempered by keeping at room temperature for 1 hr, until the core temperature reached 0 - 2^0C. The surimi was then cut into small pieces with an approximate thickness of 1 cm. Chopped surimi placed in a chopping machine and 3% salt was added in surimi and seaweed extract (Phenolic compounds), at different concentrations i.e. 0.5%, 1.0%, 1.5%, 2.0% and 2.5% (% concentration of seaweed extract is based on total weight of surimi) were added during surimi chopping. The mixture was chopped for twenty minutes at low temperature to obtain a homogeneous surimi gel. The sol was then stuffed into krehlon casing with a diameter of 2.5 cm and both ends of the casing were sealed tightly. Sols were incubated at 40^0C for 30 min, followed by heating at 90^0C for 20 min in water bath. The control gels were prepared in similar way, without seaweed extract addition. After heating prepared surimi sausages were chilled in ice for 20 min and stored overnight in the refrigerator prior to analyses.

Measurement of Gel Properties

Textural Analysis: Textural analysis of gels was performed using a texture analyser - RHEO TEX (Type: SD-700; Japan) at room temperature. Prepared surimi sausages were cut into five cylindrical shaped pieces of 2.5 cm in length. The breaking force (gel strength) and deformation (elasticity/deformability) were measured for each sample by keeping the pieces of each sample into the texture analyzer equipped with a spherical plunger (5 mm diameter; 60 mm/min plunger speed). The probe was pressed into the cut surface of a gel specimen perpendicularly at a constant speed, until puncture occurred. The force in gram (gm) required to puncture into the gel (breaking force) and the distance (in mm) at which the ball probe punctured into the gel (deformation) were recorded. Gel strength for each surimi sausage was measured from respective breaking force and deformation.

Determination of Whiteness: Whiteness was measured by using whiteness meter (MINIOLTA, Chroma meter CR-400; Japan). The whiteness meter was standardized for whiteness by using

white plate. L* (lightness), a* (redness/greenness), and b* (yellowness/blueness) were measured and whiteness was calculated as described by Balange and Benjakul (2009b) as follows:

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

Determination of Expressible Moisture Content: Expressible moisture content was measured according to the method of Balange and Benjakul (2009b) with a slight modification. Gel sample with a thickness of 0.5 cm was weighed (X) and placed between 3 sheets of Whatman paper no. 4 at the bottom and 2 sheets on the top of the sample. The standard weight (5 kg) was placed at the top and held for 2 min. The sample was then removed from the papers and weighed again (Y). Expressible moisture content was calculated using the following equation:

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

Determination of pH: pH was measured by using pH meter (Sentex, USA). For which, surimi sausage 5 gm sample was weighed and ground with 45 ml distilled water and filtered using a filter paper. The pH of filtrate was recorded by using a pH meter.

SDS-Polyacrylamide gel electrophoresis: Protein patterns of surimi gels added with or without seaweed extract at various concentrations were analysed by SDS–PAGE according to the method of Laemmli (1970). To prepare the protein sample, 27 ml of 5% (w/v) SDS solution heated to 85^{0}C were added to the sample (3 gm). The mixture was then homogenized using a homogeniser (Polytron, Kinematica, Switzerland) at a speed 11,000 rpm for 2 min. and incubated at 85^{0}C for 1 h to dissolve total proteins. The samples were centrifuged at 3500×*g* for 20 min to remove undissolved debris. Protein concentration of the supernatant was determined by the Biuret method (Robinson and Hodgen 1940) using bovine serum albumin as a standard. The sample was then mixed with sample buffer (4 ml of 10% SDS, 2 ml of glycerol, 1 ml of β-mercaptoethanol, 2.5 ml of 0.5 M Tris–HCl (pH 6.8), and 0.03 gm Bromophenol blue) at 1:1 ratio (v/v). The samples (20 µg protein) were loaded onto the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Hoefer Mini Electrophoresis (San Francisco, USA). After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

Statistical Analysis: The experiments were run in triplicate. Analysis of variance (ANOVA) was performed and the mean comparisons were carried out by Duncan's multiple range tests (Steel and Torrie 1980). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 10.0 for windows: SPSS Inc., Chicago, IL). The statements of statistical significance were based on $P<0.05$.

RESULTS AND DISCUSSION

Effect of seaweed extract on breaking force and deformation of mackerel surimi gel: Mackerel surimi gels were prepared with 0%, 0.5%, 1.0%, 1.5%, 2.0% and 2.5% (of total weight of surimi) seaweed extract concentrations containing total phenolic compound of 16.24 mg tannin / gm of dry seaweed powder. Surimi gel with 1.5 % seaweed extract had the significant ($P < 0.05$) increase in breaking force up to 128 gm, compared with that of the control with gel strength of 106 gm (Fig 1a). For gels added with 0.5%, the minor increase in breaking force was observed compared with that of the control. Progressive increase in both breaking force and deformation was observed when dry seaweed extract concentrations were used from 0.5 to 2.0% (Fig. 1b). Use of dry seaweed extract at 2% concentration, increased breaking force of mackerel surimi gel by 20.60% and deformation by 9.90% compared with breaking force and deformation of control surimi gel.

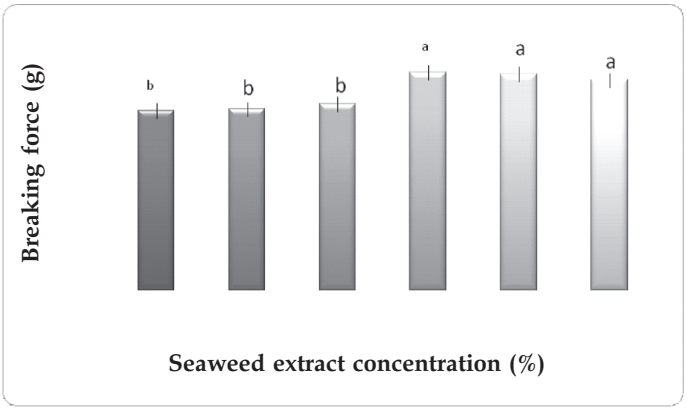

Figure 1a : Effect of dry seaweed extract on breaking force of mackerel surimi gel

[a,b] Bars with different letter differ significantly ($P < 0.05$), n = 3)

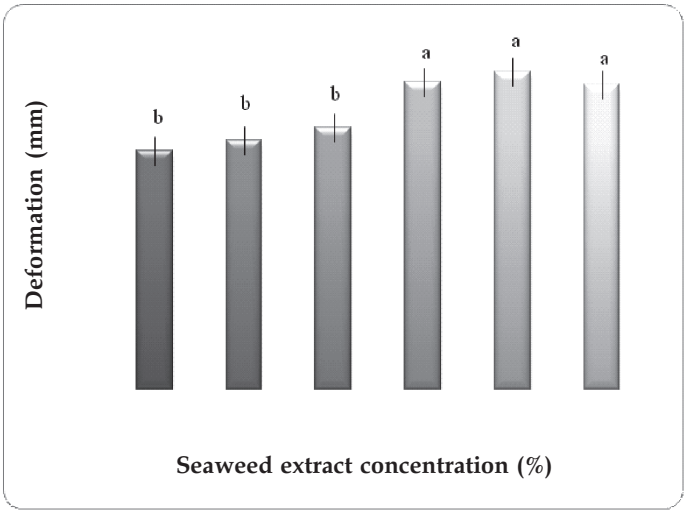

Figure 1b : Effect of dry seaweed extract on deformation of mackerel surimi gel

[a,b] Bars with different letter differ significantly ($P < 0.05$), n = 3)

Mackerel surimi gel prepared with addition of 1.5 and 2% dry seaweed extract showed significant increase in breaking force and deformation as compared with control. This increase in gel strength might be attributed to the cross-linking activity of phenolic compounds present in the seaweed extract which could induce the formation of both covalent and non-covalent bonds of gel matrix (Prigent *et al*. 2003). The result is in accordance with that of Balange and Benjakul (2009b) who reported the increases in breaking force and deformation of mackerel surimi with the addition of kiam wood extract containing phenolic compound. In the present study, the decrease in gel strength of surimi gel with increasing concentration of dry seaweed extract may be associated with self-aggregation of phenolic compounds, leading to the loss in capability of protein cross-linking. Freitas and Mateus (2001) found that the high concentration of phenolic compounds lower efficiency in interacting with protein.

Effect of seaweed extract on expressible moisture: Significantly lower ($P<0.05$) expressible moisture content of mackerel surimi was found when seaweed extract at optimum level (2%) was added (Figure 2). There is no significant difference between 1.5 and 2% level. At the optimal level, the cross-linking of proteins in the mackerel surimi gels could be enhanced, which resulted in the formation of stronger network with greater water holding capacity. Among the extracts WSE at a level of 2.0 % yielded the gel with the lowest expressible moisture content. This reconfirmed that WSE addition resulted in gel strengthening. As a result, gel network with capability of imbibing water could be obtained. Balange and Benjakul (2009b) also reported lowest expressible moisture content of mackerel surimi gel prepared with 0.30% water kiam wood extract. Also, increase in expressible moisture content was found in surimi gels added with water kiam wood extract above optimum level. Lowest expressible moisture content in gels with addition of 0.50% oxidized tannic acid was reported by Balange and Benjakul (2009c).

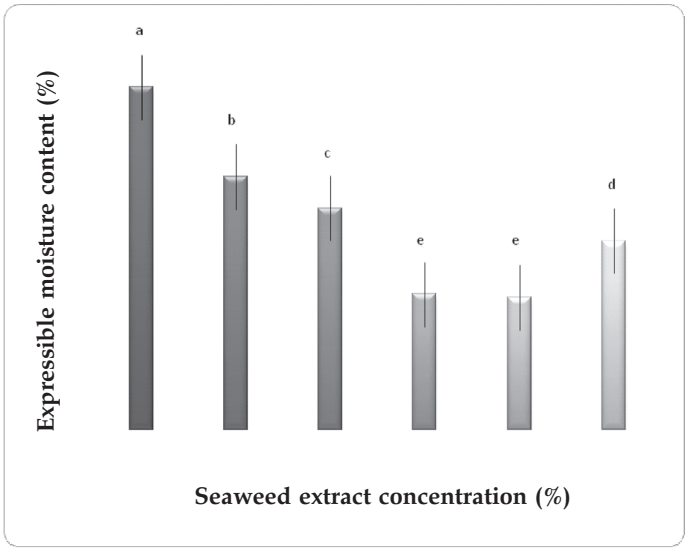

Figure 2 : Effect of dry seaweed extract on expressible moisture content of Mackerelsurimi gel

[a,b] Bars with different letter differ significantly ($P < 0.05$), n = 3)

Effect of seaweed extract on whiteness of mackerel surimi gel: Decrease in whiteness of mackerel surimi gels was observed with increase in seaweed extract concentration and the maximum reduction was observed due to 2.5% addition of seaweed extract (Figure 3). From the observations, it was seen that control surimi gel had 48.28 whiteness and it decreases up to 44.55 in surimi gel prepared with 2.5% seaweed extract. Evaporation of water extract at high temperature for a long time enhanced the darkening of water extract. Pansera *et al*. (2004) used the process of hydrosolubilisation at 100^0C for the extraction of tannin and found that the extraction process at high temperature motivates a hydro cracking of sugar and other organic compounds with darkening of the final product.

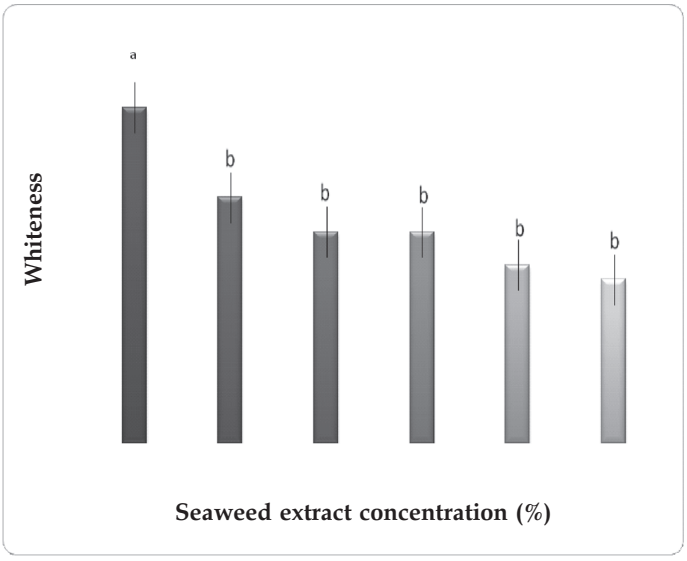

Figure 3: Effect of dry seaweed extract on mackerel surimi gel whiteness

[a,b] Bars with different letter differ significantly ($P < 0.05$), n = 3)

Effect of seaweed extract on the pH of mackerel surimi gel: In the present study, addition of dry seaweed extract containing phenolic compounds showed insignificant increase in pH ($P > 0.05$) with increasing concentration of seaweed extract in surimi gel. pH observed in surimi gel prepared without seaweed extract was 6.65 and with 2.5% seaweed extract it was 6.65. The dry seaweed extract added in mackerel surimi gel contained higher amount of phenolic compounds i.e. polyphenols, possessing one or more aromatic rings bearing hydroxyl substituent (Parr and Bolwell 2000), which might have resulted into slight but insignificant increase in pH of surimi ($P>0.05$).

Effect of Seaweed Extract on the protein pattern of Mackerel surimi gel: The protein patterns of surimi gels without and with the addition of seaweed extract are depicted in Fig 4. For gels with addition of 1.5 and 2.0% seaweed extract, the MHC

band disappeared slightly. The result suggested that MHC was cross-linked by phenolic compounds to some extent via non-disulphide covalent bonds. MHC was most susceptible to cross-linking during setting (Benjakul *et al*. 2004a). These results are in accordance with the increased breaking force and deformation and less expressible moisture when seaweed extract at 1.5 and 2% were added into mackerel surimi. No marked changes in actin band intensity were observed between the control gel and those with addition of different concentration of seaweed extract. Additionally, dark flesh fish were reported to possess high autolytic activity (Benjakul *et al*. 2004b), which is associated with the poor gel properties. It was postulated that phenolic compounds might partially lower the proteolysis caused by endogenous proteinases. Cross-linked proteins were more likely less susceptible to proteolysis. This might be associated with gel strengthening in addition to enhanced protein cross-linking in the present investigation.

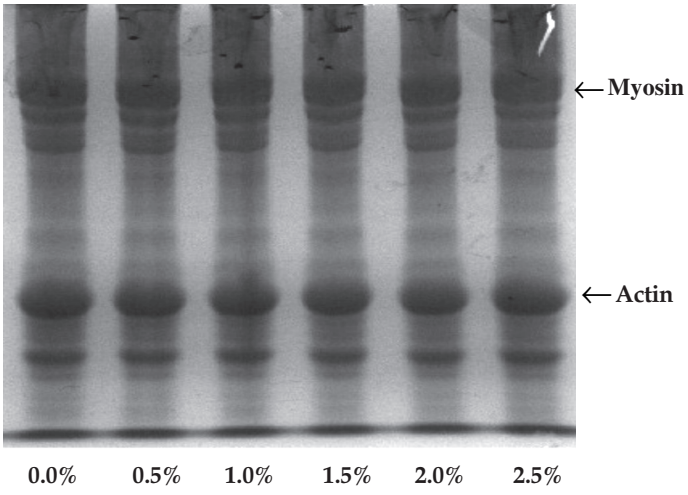

Figure 4 : Effect of dry seaweed extract on the protein pattern of Mackerel surimi gel

CONCLUSION

Seaweed extract had a potential in strengthening the gel of mackerel surimi when 1.5 or 2% level were added. Addition of seaweed extract had no detrimental effect on sensory properties of surimi gel. Thus, the extract from seaweed can be used as a natural gel enhancer for mackerel surimi industry.

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