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Original article

Influence of histidine on gelation properties of low sodium surimi from tilapia (*Oreochromis niloticus*)

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Summary The aim of this study was to reduce the salt content of tilapia surimi using histidine without affecting gelation properties. The addition of histidine (T-1) to low salt surimi gels increased the breaking force and deformation and resulted in the highest gel strength (458 g.cm). Expressible moisture was less in the T-1 sample and proteolytic degradation was higher in the control sample indicating that autolysis did not occur in histidine added sample during gel setting. Protein patterns revealed cross-linking of myosin heavy chain in gels with added histidine. Fourier transformed infrared spectra implied that histidine-induced unfolding of proteins occurred after heating. T-1 samples exhibited a dense and compact microstructure, whereas the control gel was loose. The results of this study show that the addition of histidine could yield high-quality gels from tilapia surimi in low salt conditions.

Keywords FTIR, gel strength, histidine, imidazole ring, low salt surimi.

Introduction

Low sodium meat products have gained popularity in recent years as people become more aware of their food intake and its impact on their health, and they strive to eat wholesome foods. The major quantity of sodium in the diet is obtained through processed foods, either because they have a high salt content or because they are consumed often and in significant quantities. In addition, during the cooking process also salt is added to the food (Ni Mhurchu *et al*., 2011). Sodium chloride is a common ingredient in meat processing that affects the shelf life, texture and enhance the flavour of meat products by bringing out its unique taste (Ruusunen & Puolanne, 2005). Excessive dietary salt intake has been shown to create short-term symptoms such as swollen face, swollen hands and feet and in extreme cases elevates blood pressure, which is the leading cause of cardiovascular disease (CVD) (Gelabert *et al*., 2003). According to the World Health Organization, most people consume too much salt (9–12 g per day), which is approximately twice the recommended maximum intake (5 g per day). In many countries, processed foods account for around 80% of salt in the diet (WHO, 2002). Therefore, the most effective way to prevent salt-related cardiovascular diseases is to limit sodium at the food manufacturing level.

Most of the seafood products, especially surimi and related products, are among the world's most popular foods today and have a distinct place in stores. The texture of surimi products is greatly influenced by sodium chloride. It enhances the fat and water-binding capacity of surimi products by extracting myofibrillar protein, as a result of which a desirable gel texture is formed during cooking (Choi *et al*., 2014). Consumers are now more aware of consuming salty diet and associated health consequences. As a result, by focusing on this market, processors will be able to diversify their product lines by developing low-sodium surimi-based fish products. However, reduction in salt in the surimi is not an easy task for the processors as salts play such an important role in surimi production. Several studies have attempted to substitute other cations, primarily KCl, for NaCl in meat products (Soglia *et al*., 2014; Yu *et al*., 2017). However, addition of KCl into the meat products can induce bitterness and reduce the saltiness of the foods, which is a major drawback for using potassium chloride rather than sodium chloride.

In this study, histidine (His) was used to increase the gelation properties of surimi containing less quantities of salt by solubilising the proteins. Histidine is a

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basic amino acid but it can have a positive or a neutral charge on its side-chain group at the physiological pH and its structure consists of L-α-alanine and an imidazole ring (Chen *et al*., 2016a). Histidine could aid to the salty taste (Zhang *et al*., 2015) and is being utilised as a salt replacement component in surimi products. Histidine were found to induce the solubility of chicken and porcin myosin (Hayakawa *et al*., 2009; Guo *et al*., 2015). Nevertheless, histidine as a salt substitute components have not been used in tilapia surimi. Therefore, the aim of the present investigation was to study the effect of histidine on the gelation properties of tilapia surimi gels containing lesser amount of salt.

Materials and methods

Raw material

Around 25 cm length and 750 g weight of tilapia (*Oreochromis niloticus*) was procured from West coast fine foodstuff LLP, Mumbai, Maharashtra, India, and carried to the laboratory on ice within 30 min. Transported fish were instantly washed with cold water and subsequently headed, eviscerated and completely washed, and deboning was done with a mechanical deboner (Baader 694, Lubeck, Germany) that had a drum consisting of 5-mm-diameter hole and rotating belt.

Preparation of surimi

Surimi was prepared by single washing of fish mince by mince/water ratio of 1:3 (w/v) using cold water (4 °C) as described by Priyadarshini *et al*. (2017). The mixture was gently mixed for 3 min in a Hobart mixer (Hobart AE 200, London, England) and left to settle for 2 min after being washed with cold water. A double-layer muslin cloth was used to strain the slurry, and the extra water was squeezed out manually. After that, the mince was subjected to centrifuge in a basket centrifuge for 15 min at 100 g (Model 60-5, AIM Industries, Mumbai, India). The surimi which is prepared was packed in low-density polyethylene pouches and stored in ice for immediate use in surimi gel preparation.

Preparation of tilapia surimi gel

The surimi was chopped for 2 min by using silent cutter operated at low speed. A uniformly mixed surimi sol was obtained by adding different concentration of salt and amino acid. The sol mixed with 1% salt (Control), 1% salt and % 0.9% histidine (T-1) was previously optimised by response surface methodology (RSM). The mixed paste was stuffed into PVDC casings, which had a length of 17.5 cm and diameter of 2.5 cm, by using sausage stuffer (HK1280, Electomania, China) and both the side of the casings were tied with a thread. The gels were subjected to heating for 30 min at 40 °C, followed by 20 min at 90 °C. Finally, the gels were cooled in ice and refrigerated at 4 °C for overnight before being analysed.

Assessment of gel strength

Gels were removed from the PVDC casings and sliced into 2.5-cm-long cylinder-shaped samples for determining the gel strength after around 2 h of equilibration at room temperature (25 °C). The puncture test was performed on gel samples using a Rheo Tex (Type SD-700, Sun Scientific co. ltd, Japan) outfitted with a metal probe of 5 mm diameter with 2 kg load cell. During compression, the gel got ruptured and lost its strength, which gives the breaking force and deformation (depth of depression) of the sample. All of the tests were performed in duplicate.

Texture profile analysis of gel

Texture analysis was carried out using texture analyser (Texan touch, Lamy Rheology, France) for each treatment groups. The cylindrical gel sample was cut into 2.5 cm and kept on the flat platform of the texture analyser. The sample was compressed twice to 40% height of the sample. Hardness, cohesiveness, elasticity and adhesiveness were determined from the recorded force–time curves using 50-mm-diameter cylindrical probe made up of stainless steel.

Expressible moisture content (EMC)

A gel sample was cut into 0.5 cm thickness, approximately weighed (X g) and kept in between the three parts of Whatman filter papers No. 93 at the top and bottom. At the top of the sample, the standard weight of 5 kg was placed and remained for 2 min. The samples were taken out of the paper and weighed again (Y g). The expressible moisture content was calculated using the equation below (Balange & Benjakul, 2009).

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

Determination of whiteness of gel

Hunterlab (ColorFlex, Hunter Associates Laboratory, Reston, VA) was used to measure the colour parameters of surimi gel. The colour parameters such as lightness (L^*), redness (a^*) and yellowness (b^*) were measured and the following equation was used to calculate whiteness (Javith *et al*., 2020).

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

TCA-soluble peptide content

About 2 g of sample was mixed with 18 mL of TCA (5%). The blend was homogenised (Polytron PT2100, Kinematica AG, Switzerland) at a speed of 13,500 g for 2 min. The homogenate was kept for 1 h at 4 °C and centrifuged (Elektrocraft Pvt. Ltd., India) at 8000 ***g*** in room temperature for 5 min. The supernatant containing TCA-soluble peptide was measured in accordance with the method described by Lowry *et al.* (1951). Tyrosine was used as a standard (μmol tyrosine per g of sample).

Fourier Transform Infrared Spectroscopy (FTIR)

Secondary structure of protein in low salt surimi gel was analysed using FTIR spectroscopy (Model: 3000, Ettlingen, Germany) provided with a micro-attenuated total reflectance (ATR) accessory. The sample (1 mg) is mixed with potassium bromide (KBr) at a ratio of 1:100 and consequently formed into a matrix. The matrix was pressed into a thin disc using a hydraulic press. The capturing of spectra was done by opus Software version 6.5. The wavenumber ranged from 4000 to 400 cm^{-1}. At a resolution of 4 cm^{-1}, an average of 32 spectro scans was collected. The spectra were recorded in transmittance mode.

Protein pattern by using SDS-PAGE

SDS-PAGE electrophoresis was used to analyse the protein pattern of the surimi gel (Laemmli, 1970). The samples (15 μg protein) were loaded into the polyacrylamide gel consisting of running gel (10%) and stacking gel (4%), and electrophoresis was performed on each gel at a constant current of 15 mA. The gels were stained and destained after separation and the picture was scanned by using scanner.

Microstructure of surimi gel

Scanning electron microscope (SEM) was used to observe the microstructure of surimi gel. Samples (2–3 mm) were fixed with 2.5% glutaraldehyde solution and washed with distilled water for 1 h followed by dehydration using series of ethanol concentration (25%, 50%, 70%, 80%, 90% and 100%) for 10 min each. For critical point drying, CO2 was used as a transition fluid. The dried samples were mounted on a bronze stub and sputter coated with gold. The specimens were examined using a SEM (JEOL JSM-6390 LV, Tokyo, Japan) in the Environmental SEM mode at a 15 kV acceleration voltage.

Statistical analysis

Statistical analysis was done by using statistical Package for Social Science (SPSS 16.0 for windows). The data were reported as average values ± standard deviation (SD), and to assess the statistical significance ($P < 0.05$) between the triplicates, *post hoc* comparison was performed (Steel & Torrie, 1980) using Duncan's multiple-range test.

Results and discussion

Determination of Gel strength

The quality of the surimi gel can be assessed by measuring breaking force (g) and deformation (cm) of the surimi gel. There was a significant difference ($P < 0.05$) in gel strength between control and His (T-1) added sample (Table 1). From the results, it was observed that T-1 (458 g·cm) sample had significantly ($P < 0.05$) higher gel strength than the control (181 g·cm). Protein gelation is initiated from solubilisation of myofibrillar protein by salt followed by aggregation during heating. Lowest gel strength in the control sample is due to the poor solubilisation of myofibrillar protein in the presence of low salt. The low solubility of myofibrillar protein at low ionic strength is mainly due to the formation of myosin filaments. The gel strength was improved significantly ($P < 0.05$) with the addition of histidine since His could lead to change the conformation of native myosin resulting in the loosening of the myosin filament in myofibril. Hayakawa *et al.* (2009) reported that the elongation of the myosin rod in a low ionic strength solution containing His would inhibit the formation of a filament, resulting in the solubilisation of myosin. Once the myofibrillar protein gets solubulised by His, consequently aggregation of myosin heads occurred to form a stronger gel network during heating. In addition, gelation of the surimi occurs through cross-

Table 1 Effect of histidine on Gel strength, Whiteness, EMC and TCA-SPC of surimi gel

Parameters	C	T-1
Breaking force (g)	245 ± 14.65[a]	522 ± 12.79[b]
Deformation (cm)	0.74 ± 0.04[a]	0.88 ± 0.04[b]
Gel strength (g·cm)	181 ± 14.85[a]	458 ± 22.01[b]
Whiteness	71.45 ± 0.86[a]	67.26 ± 1.04[b]
EMC (%)	13.15 ± 0.77[a]	2.83 ± 0.02[b]
TCA-soluble peptide content (μmol tyrosine per g sample)	3.79 ± 0.05[a]	2.40 ± 0.04[b]

Values are mean ± SD ($n = 3$). Different superscripts in the same column denote significant differences ($P < 0.05$).
C – Control (1.0% salt), and T-1 – 1% salt and 0.9% histidine.

linking of Ɛ-(*type="InGreek_and_Coptic"*>ϒ-Glu)-Lys linkages and myosin heavy chain during the heating process (Araki & Seki, 1993). The present finding was in concordance with the report of Gao *et al.* (2021); they reported increasing gel strength of Amur sturgeon surimi containing reduced salt and histidine. Similar results were observed in big head carp myosin (Gao *et al.*, 2018).

Texture profile analysis

Texture profile includes a number of attributes such as hardness, cohesiveness, adhesiveness and springiness. Significant ($P < 0.05$) difference was observed in the hardness value between control and amino acid-treated sample. T-1 sample had significantly ($P < 0.05$) higher (63.72 N) hardness than the control (40.14 N). These results are in agreement with the gel strength values (Table 1). The present results indicate that when compared to control, the hardness value of the samples increased effectively by improving the cross-linking induced by histidine. In the present investigation, there was no significant difference ($P > 0.05$) observed in cohesiveness, adhesiveness and elasticity values between control and T-1 sample (data not shown).

Expressible moisture content

Expressible moisture content (EMC) is negatively related to the water-holding capacity (100-WHC). The WHC denotes the water trapping capability of the protein matrix that is related to the yield and quality of the surimi and related products. Low salt surimi gel with or without addition of His showed significant difference ($P < 0.05$) in the EMC (Table 1). Lower EMC was found in T-1 sample than the control. These findings were in agreement with the gel strength of the sample and also indicate the capability of the gel to hold more water inside the stronger protein network caused by the addition of His and the same is evident with lower EMC in T-1 sample in the present investigation. The decrease in EMC associated with the increased gel strength of the low sodium surimi was induced by addition of His (Gao *et al.*, 2019). Similarly, the microstructure of the gel network had a significant impact on the WHC of myofibrillar protein gels (Wang *et al.*, 2021). The WHC is increased in heat-induced protein gels when a dense network structure traps more water via hydrogen bonds (Xia *et al.*, 2019).

Changes in whiteness

Whiteness is one of the prime characteristics of the surimi and related products. There was a significant ($P < 0.05$) difference in whiteness value in the His added sample compared to the control (Table 1). The result shows higher whiteness in control sample (71.11) than T-1 (67.26) sample, probably due to greater dispersion of light in low salt surimi sample. Incomplete protein solubilisation could be related to a non-uniform network, which exhibited a greater light dispersion influence on the gel, particularly at the surface (Buamard *et al.*, 2020). Hong *et al.* (2006) stated that the moisture content of the sample will influence the lightness (L^*) of the product. The whiteness index is positively correlated with the lightness (L^*) value. Addition of histidine resulted in the decrease in expressible moisture content (EMC), which indicates the presence of higher moisture content, therefore the T-1 sample had significantly ($P < 0.05$) lower whiteness than the control. Similarly, the present results were compared with an earlier study of Gao *et al.* (2021), wherein they reported that colour of amur sturgeon surimi gel was significantly decreased ($P < 0.05$) after the addition of histidine which have antioxidant activity and coordination capability with endogenous metallic ions, preventing ferroporphyrin oxidation and causing an increase in a^* values and a decrease in b^* values.

TCA-Soluble peptide content

TCA-Soluble peptide content (TCA-SPC) of surimi gel was analysed to find out the degradation of protein by endogenous proteases during thermal setting and gelation. Significantly ($P < 0.05$) higher TCA-SPC was found in the control than T-1 sample (Table 1) which indicates that enhanced hydrolysis of protein occurred in the control sample by endogenous protease (Rawduken *et al.*, 2009). This finding was in accordance with the minimal gel strength of control (Table 1). The present results also suggest that the addition of histidine could inhibit the muscle protein degradation which in turn enhances the gel strength by protein cross-linking. Histidine can form hydrogen bonds with polar and charged residues as well as interact with other aromatic residues (Doytchinova, 2014). As a result, the extraction of smaller peptide may be hindered as it was indicated by the lesser amount of TCA-soluble peptides.

Protein pattern of surimi gel

Protein pattern of unwashed mince, surimi and gels made from low salt surimi are shown in Fig. 1. Myosin and actin is the major fraction of the myofibrillar protein, which could be solubilised in low ionic strength solution containing histidine. The myosin heavy-chain (MHC) band intensity was decreased in T-1 sample compared to control, unwashed mince

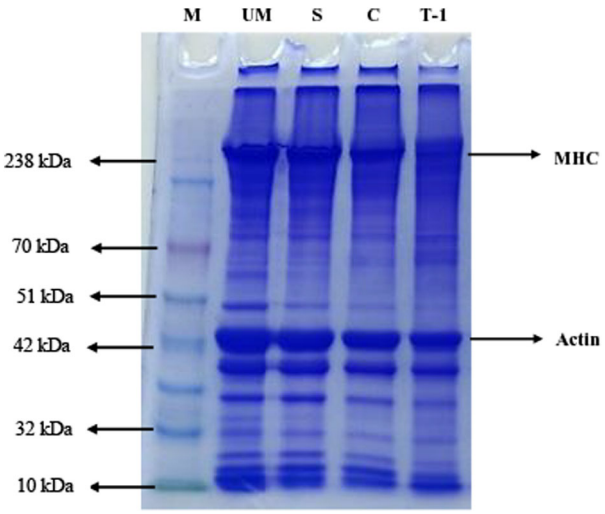

Figure 1 Effect of histidine on protein pattern of surimi gel. M – Protein marker, UM – Unwashed mince, S – Surimi, C – Control (1.0% salt), T-1 – 1% salt and 0.9% histidine.

(UM) and surimi (S). However, slight fading of MHC band intensity was noted in control sample compared to unwashed mince and surimi. The disappearance of MHC band intensity is mainly caused by cross-linking of protein which is accelerated by endogenous transglutaminase (TGase). The actin band did not disappear and its band intensity also remained same for all the gels indicating that actin could not be polymerised during gelation as well as more resistant to proteolysis.

Changes in secondary structures

FTIR spectroscopy was used to analyse the secondary structure of protein. FTIR spectra provides peak from wavenumber 4000 to 400 cm^{-1} (Fig. 2) which represents the various interaction of the compound. Generally, amide I region represents secondary structure of protein such as α-helix, β-sheet, β-turn and random coil structure which varies from 1600 to 1700 cm^{-1} (Bertram *et al.*, 2006). Changes in the amide I band is mainly caused by the reduction in α-helix to form a β-sheet which has been utilised to interpret the mechanism of gelation. The peak at 1653 cm^{-1} and 1652 cm^{-1} in unwashed mince and surimi was related with C = O in an α-helical structures of protein. In the control gel, the characteristic peak was observed at 1648 cm^{-1} represents the formation of random coil structures which indicates the initiation of protein denaturation during thermal setting (Priyadarshini *et al.*, 2017). In T-1 gel, the peak was found at 1632 cm^{-1} which is likely due to reduction in α-helix structure of native myosin, indicating that unfolding of protein after heating induced by His containing imidazole ring leads to formation of β-sheet structure. As the α-helix structure of myosin was primarily stabilised by hydrogen bonds between the carbonyl oxygen (ACO) and amino hydrogen (NHA) of the polypeptide chain, the presence of nucleophilic centres in the imidazole ring may have disrupted these hydrogen bonds, changing the structure of the molecule. The present result is consistent with the report of Chen *et al.* (2016a), who observed that addition of different chemical constituent of His-containing imidazole ring

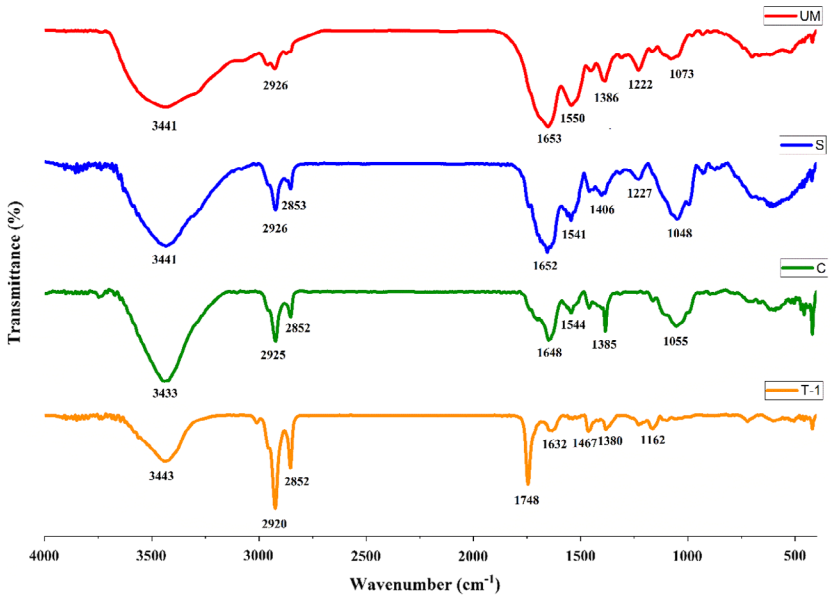

Figure 2 FTIR spectral image of surimi gel. M – Unwashed mince, S – Surimi, C – Control (1.0% salt), T-1 – 1% salt and 0.9% histidine.

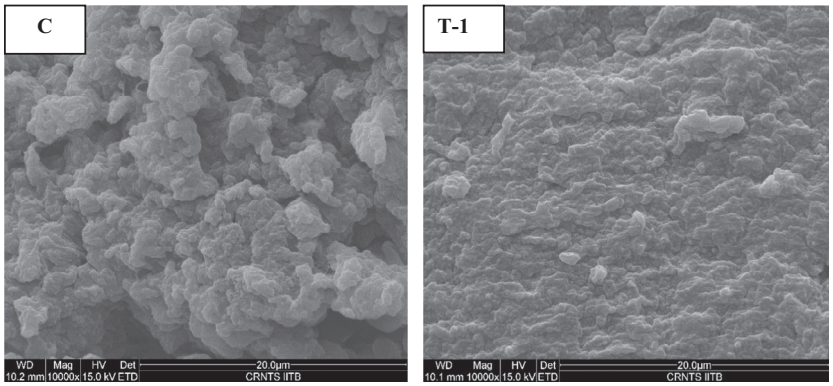

Figure 3 Effect of histidine on microstructure of surimi gel. C – Control (1.0% salt), T-1 – 1% salt and 0.9% histidine.

reduces α-helix structure of protein. Guo *et al.* (2015) observed a similar reduction in α-helix and increased another secondary structure, especially random coil, when histidine is added to porcine myosin. Histidine cations can bind negatively charged myosin residues by electrostatic interaction, interrupting intra- and intermolecule ionic bonds and causing myosin conformation to change, resulting in the loss of α-helical structure and the exposure of hydrophobic, and masked SH groups to the surface.

Microstructure of surimi gel

Microstructures of low salt surimi gel added with and without His are shown in Fig. 3. The control gel had a coarser and loose protein network with the presence of larger voids or cavities. Looser network is associated with the partial solubilisation of proteins, mainly due to a lack of salt content. T-1 sample had a denser and compact protein network without the presence of voids. As discussed above, His could effectively solubilise the protein resulting in unfolding of myosin and also expose the reactive groups which interact with each other to form protein aggregates and uniform compact gel network structure during heat setting mainly through hydrophobic interaction. Microstructure is mainly contributed to the WHC and gel strength. His induced dense networks may be able to hold more water and thus promote better water-binding capacity as demonstrated by decreased EMC (Table 1). The findings were consistent with earlier study of Hayakawa *et al.* (2012) and Chen *et al.* (2016b), where they reported fine network structure of chicken breast myofibrillar protein with the addition of His resulting from the low hydrophobicity and non-decreasing reactive SH content in myosin head interactions as well as structural changes in myosin rod portion (Hayakawa *et al.*, 2012).

Conclusion

The result from the present investigation revealed that the incorporation of histidine could effectively enhance the gel properties of tilapia surimi in low salt condition. Incorporation of His in tilapia gel exhibited highest gel strength and textural properties. However, surimi gel without His showed higher whiteness when compared to His added surimi gel. FTIR and SEM results revealed that His added surimi can produce strong network gel through proper unfolding of protein followed by aggregation during setting. Therefore, His can be used as a substitute for the salt to improve the gel properties of tilapia surimi and surimi-related products.

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Ethical guidelines

Ethics approval was not required for this research.

Conflict of interest

All the authors declare no conflict of interest to publish this work.

Author contribution

Mohammed Akram Javith S: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Methodology (lead); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review & editing (lead). **Janarthanan Gunasekaran:** Data curation (supporting); Formal analysis (supporting); Methodology (supporting); Writing – review & editing (supporting). **MARTIN XAVIER:** Data curation (equal); Validation (equal); Writing – review & editing (equal). **Binaya Bhushan Nayak:** Supervision (equal); Validation (equal). **Gopal Krishna:** Resources (equal);

Supervision (equal). **Amjad K. Khansaheb Balange:** Conceptualization (equal); Data curation (equal); Investigation (equal); Methodology (equal); Resources (equal); Supervision (equal); Validation (equal); Visualization (equal); Writing – review & editing (equal).

Peer review

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Data availability statement

The research data are not shared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Effect of histidine on texture profile analysis of surimi gel.