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Exploration and Evaluation of Pony Fish (*Leiognathus* sp) Mince as a Source of Surimi and Study its Spectral and Textural Attributes

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ABSTRACT

Background: This is the first study in Indian context where underutilized fish species pony fish (*Leiognathus* sp.) has been used for the preparation of surimi and surimi gel and its textural and spectral images were analysed.

Methods: Textural profile analysis for estimating the hardness, gumminess and chewiness parameters and colour analyser for measuring the difference in whiteness among samples were used. FTIR (Fourier Transform Infrared Spectroscopy) was used to estimate the percentage of proteins folded during heat denaturation process.

Result: Textural parameters of CWS (Conventional Washed Surimi) gel showed ideal gel strength of 191.06 g cm, hardness of 46.48 N, gumminess of 30.75 N and chewiness of 28.70 N. Colour analysis of UM (Unwashed Mince), CWS and CWS gel showed whiteness of 38.59, 61.45 and 72.07. FTIR analysis of UM, CWS and CWS gel shows unfolding of protein structures in the wavelength of 1688.07 cm^{-1}, 1665.34 cm^{-1} and 1653.20 cm^{-1}. This study reveals the potential use of pony fish (*Leiognathus* sp.) as one of the favorable fish species for surimi and sausage production showing virtuous instrumental quality attributes.

Key words: FTIR, Gel strength, Pony fish surimi, SEM, Texture, Whiteness.

INTRODUCTION

Fish is one of the staple proteinaceous foods consumed across different countries in the form of fresh, frozen, canned, dried, salted and other value added products. One of the viable processing methods with which excess fish catch can be preserved for long time in the food industries in the form of ground meat or wet myofibrillar concentrate and utilized for the preparation of various products like kamabako, fish sausages, value added products *etc.*

The most commonly used species in India for the preparation of surimi are Mauve-lip thread-fin bream (*Nemipterus mesoprion*, Bleeker, 1853), Brown spotted grouper (*Epinephelus chlorostigma*, Valenciennes, 1828), Large head hairtail (*Trichiurus lepturus*, Linnaeus, 1758), Big-eye snapper (*Priacanthus* spp.) and Lizardfish (*Saurida* spp.) (Muraleedharan *et al*., 1996). In order to overcome the shortage of major raw materials, underutilized fish species like pony fish (*Leiognathus* sp.) could be effectively used as a raw material for surimi industries (FAO 2016; CMFRI 2019 Annual Report). Pony fish or silver bellies which is a main discard linked with trawl by-catch cost around Rs. 20-25/kg (USD-0.27-0.34) which is mostly utilized as a major protein source for poultry feed industries and having potential to substitute commercial fish species used in surimi industries for the preparation of gel based products and there is potential market for expediency products from this fish species.

However, limited reports were available for the utilization of low value fishes like sardines, catfish and lizard fish into surimi products and no previous study was reported from pony fish (*Leiognathus* sp.) in Indian context. Hence, the development of converting discards of low value fish to a marketable product is required and based on the information available, this is the first study in India, aims the potential use of pony fish (*Leiognathus* sp.) as a source of surimi and its significant qualitative and quantitative importance in fulfilling the requirements of surimi industries.

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MATERIALS AND METHODS

100 kg of fresh fishes (*Leiognathus* sp.) with standard length of 16.8±4.60 cm each were procured from palk bay region of Tamil Nadu, India and brought to the Post Harvest

technology laboratory, Central Institute of Fisheries Education, Mumbai and the entire work was carried out during the year 2019 to 2021. The fishes were kept under iced condition until it is used for surimi preparation.

Preparation of mince

Prior to the preparation of Surimi, the fishes were washed, gutted, cleaned and filleted.

Flow chart for the preparation of fish mince is described below:

Whole fishes
↓
Washed with potable water to remove slime, dirt, blood *etc.*
↓
Gutted and beheaded
↓
Washed with potable water and filleted
↓
The fillets were fed into deboning machine of 5 mm perforation
↓
The mince obtained was collected, weighed and packed in polyethylene pouches and stored at 4°C for surimi preparation

Fillets were subjected to deboner using a mechanical deboning machine (Baader 694, Lubeck, Germany) with a counter rotating belt and drum mechanism having perforation of 5 mm diameter and yield of mince was about 35%.

Preparation of conventional washed surimi and surimi gel

The deboned mince which was immediately used for surimi preparation is referred to as Unwashed Mince (UM). UM was washed with cold water (4°C) using a water/mince ratio of 3:1 (v/w) according to the method of Rawdkuen *et al*., (2009). The mince obtained was referred to as "Conventional washed surimi (CWS)". The UM and CWS obtained were ground for 2 min using Food Processor (Philips, Mumbai, India). NaCl (2.5%, w/w) was then added and the mixture was chopped for 1 min at 4°C to obtain the homogenous paste. The paste was then stuffed immediately into polyvinylidine sausage casing (diameter: 2.5 cm, length: 17.5 cm) with both ends of casing were sealed tightly. Two-step heated gels were prepared by setting the paste at 40°C for 30 min, followed by heating at 90°C for 20 min in a temperature controlled water bath (Strike 300, Steroglass, Perugia, Italy). Both UM and CWS gels were then cooled in iced water and stored in refrigerator for overnight at 4°C prior to analysis.

Determination of gel strength and texture profile

UM and CWS gels were taken out from synthetic casing and cut into 2.5 cm in length of cylindrical shape and were subjected to the puncture test using a Rheo-Tex (Type-Sun Rheo TEX SD-700II, Sanjo, Japan) equipped with a 5 mm-diameter round-ended metal probe (5 mm diameter, 60 mm/min) with load cell of 2 kg. The load (as breaking force, g) and the depth of depression (as deformation, cm) when the gel sample lost its strength and get ruptured were recorded. The Textural Profile Analysis (TPA) of CWS gel was performed on a TA.XT PLUS Texture Analyser (Stable Microsystem, UK software TA.XT PLUS) equipped with a stainless steel compression platen of 75 mm diameter. The CWS gels were cut into a cylinder with a diameter of 20 mm and height of 25 mm and compressed at compression degree of 40% with the pre-test, test and post-test speed of 2 mm/s, 1 mm/s and 5 mm/s.

Determination of whiteness

The Instrumental colour value was measured using Labscan XE–Colorimeter (Hunter Labscan Colour quest XE, U.S.A.) for UM, CWS and CWS gel based on L*, a*and b* values. L* (lightness), a* (redness/greenness) and b* (yellowness/ blueness) were measured and whiteness was calculated as described by Park (1994) as follows:

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

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein patterns of UM, CWS and CWS gels were determined by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis SDS-PAGE according to the method of Laemmli (1970). Samples (5 mg protein) were loaded onto polyacrylamide gels comprising a 10% running gel and a 4% stacking gel and subjected to electrophoresis at a constant current of 40 mA/gel using a Hoefer unit (Hoefer, Inc., San Francisco, CA, USA).

Fourier transform infrared spectroscopy (FT-IR)

Protein's secondary structures in UM, CWS and CWS gels were studied using a FTIR spectrometer (Model: 3000 Hyperion microscope with vertex 80 FTIR system, Bruker, Ettlingen, Germany) equipped with a micro attenuated total reflectance (ATR) accessory. 1 mg of UM, CWS and CWS gel were mixed with 100 mg of potassium bromide (KBr) and ground gently with an agate pestle and mortar under a lamp to form a very fine powder and compressed to disc using a hydraulic pellet press (Type KP, Kimaya Engineers, Mumbai, India) into a thin disc. The spectrometer was controlled by Opus Software-version 6.5 to collect spectra over the wavenumber range of 4000 to 400 cm^{-1}, by accumulating 32 scans with a resolution of 4 cm^{-1}.

Scanning electronic microscopy (SEM)

Samples of thickness 2-3 mm were fixed with 3% glutaraldehyde solution for 1 hr following the method of Liu, Zhao, Xiong, Xie and Qin, (2008) with slight modifications. The samples were then rinsed for 1 hr in distilled water before being dehydrated in a gradient ethanol series of 50, 70, 80, 90 and 100% (v/v) each 10 mins and oven dried at 50°C for 24 hrs. The dried samples were then sputter-coated with gold and observed at an acceleration voltage of 15 kV. The Microstructure of UM, CWS and gels was analyzed by using Scanning Electron Microscopy SEM (JEOLJSM-5800 LV, Tokyo, Japan).

Statistical analysis

The statistical analyses of the experiments were carried out using SPSS (Version 21.0) statistical package. The data obtained from this study were subjected to one-way analysis of variance (ANOVA) for significant differences between measured parameters and were defined at a probability level ($p<0.05$) and Duncan's multiple range tests were employed for each data group. The data are reported as mean values± standard deviation (SD). The experiments were independently triplicated (n=3).

RESULTS AND DISCUSSION

Changes in whiteness values

All the UM, CWS and CWS gels exhibited a significant difference between lightness (L*), redness (a*), yellowness (b*) values. The whiteness value exhibited a significant difference between UM, CWS and CWS gel ($p<0.05$) and the highest whiteness value found for gels prepared from CWS. UM showing lightness value of 39.08 which indicates the presence of myoglobin and other pigments. On the other hand CWS which was washed 3 times showing improved lightness value of 61.07 and redness value of -1.37. Priyadarshini *et al*., (2017) reported that the lightness value of surimi from tilapia sausage after cooking was 72.4 and the results obtained in the present investigation also showed the similar trend (Table 1). The whiteness index value of CWS was 61.45 which shows the potential of using as an alternative raw material for threadfin bream surimi which has whiteness value of 70.05 (Santana *et al.,* 2015).

Changes in gel strength values

For estimating the textural property of any surimi related products breaking force is one of the important parameter effectively analyze the quality of sausage. The breaking force and distance to rupture values of UM and CWS gels were about 69.94±4.31 and 191.06±6.26 g.cm ($p<0.05$) respectively (Table 2). The gel strength of UM was lesser than that of CWS gel which could be explained by the fact that sarcoplasmic protein in UM will coagulate by the addition of salt during the heat setting and does not take a part in the configuration of network structure whereas for CWS gel, the formation of sol takes place with addition of salt at 2.5% and ample amount of water leading to solubilisation of myofibrillar protein, which subsequently turns into an elastic gel by heating process (Lee, 1992).

Changes in texture profile values (TPA)

Texture profile analysis of CWS gel shown in Table 3. Hardness value of CWS gel shows maximum compression and deformations values (46.48 N) and was within the range (14,520-44,180 g mm, respectively) as previously reported for Malaysian commercial fish sausage (Huda *et al*., 2012). Chewiness value of CWS gel was higher which indicates more the elasticity of the product. The cohesiveness value of CWS gel samples were 0.76 N and low cohesiveness value reported might due to the addition of water and ice during sausage preparation which makes internal arrangements softer and less fragile (Dincer *et al.,* 2017). Springiness value of 0.90 mm was found for CWS gels and represents how the sausage recovered during compression tests by TPA analyzer, mainly due to the myosin contributing to the elasticity of the sausage which is the important factor in the development of kamaboko gel network (Chan *et al*., 1995).

Analyzing the protein pattern of UM, CWS and CWS gel

Protein distribution patterns of UM, CWS and CWS gels are shown in Fig 1. CWS showing clear myosin heavy chains because of augmentation of myofibrillar proteins by multiple washing methods exhibited. The visibility of sarcoplasmic proteins in gel matrix is less, indicating the successful removal of sarcoplasmic proteins during washing cycles. Heavy chain myosin bands concentration decreased in heat induced CWS gels, compared to that observed in the UM and CWS due to polymerization of protein takes place during

Table 1: Whiteness value of UM, CWS and CWS gel.

Colour values	Treatments		
	UM	CWS	CWS gel
L*	39.08±2.45^{a}	61.07±1.21^{b}	64.77±1.33^{c}
a*	1.10±0.30^{c}	-1.37±0.17^{b}	-1.83±0.12^{a}
b*	7.52±1.42^{abc}	7.47±0.42^{abc}	6.27±0.39^{abc}
Whiteness index	38.59±2.23^{a}	61.45±1.25^{b}	72.07±1.25^{c}

Data expressed as mean±Standard deviation (SD), n=3.

$^{a-c}$With mean value indicate difference between different treatments (Row).

$^{a-c}$Different superscript letters are significantly different ($P<0.05$).

UM- Unwashed mince, CWS- Conventional washed surimi.

Table 2: Determination of gel strength of UM and CWS gel.

Treatment	Breaking force (g)	Deformation (cm)	Gel strength in (g.cm)
UM	82±2.08^{a}	0.85±0.05^{b}	69.94±4.31^{a}
CWS gel	259±1.52^{b}	0.75±0.02^{a}	191.06±6.26^{b}

Data expressed as mean±Standard deviation (SD), n=3.

$^{a-b}$With mean value indicate difference between different treatments (Column).

$^{a-b}$Different superscript letter are significantly different ($P<0.05$);UM-Unwashed mince, CWS-Conventional washed surimi.

Table 3: Texture profile analysis (TPA).

Parameters	CWS gel
Hardness (N)	46.48±0.46
Adhesiveness	0.02±0.13
Springiness (mm)	0.90±0.05
Cohesiveness	0.76±0.13
Gumminess (N)	30.75±0.23
Chewiness (N)	28.70±0.23
Resilience	0.39±0.10
Stringiness	1.72±0.41

Data expressed as mean±Standard deviation (SD), n=3.

CWS- Conventional washed surimi.

setting signifying a strong gel matrix. However, minimal changes in actin were observed in UM, CWS and CWS gel because it could not be polymerized during gelation efficiently and it overcomes proteolysis effect (Balange and Benjakul, 2009).

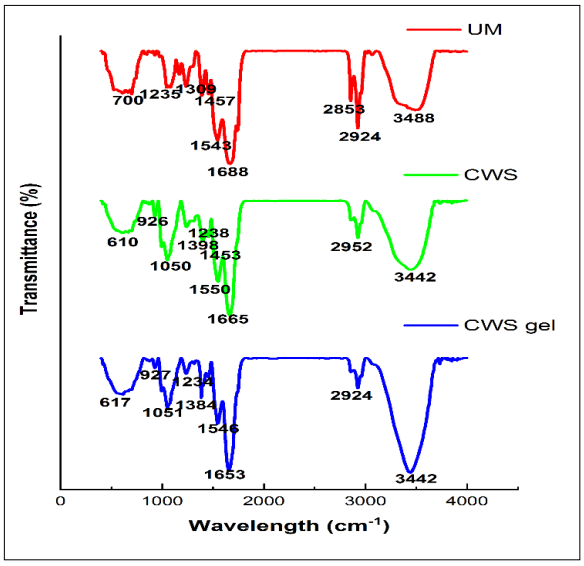

Fig 1: Fourier transform infrared (FTIR) spectroscopy of UM, CWS and CWS gel.
UM-Unwashed mince, CWS-Conventional washed surimi.

Changes in the fourier transform infrared spectra of UM, CWS and CWS gel

FTIR analytical technique extensively used for figuring the protein secondary structure. Fig 2 shows a distinctive FTIR spectrum obtained from UM, CWS and their respective CWS gel ranging from 4000 to 400 cm^{-1}. The distinctive central band for CWS at 1665.34 cm^{-1} determined for β-turn structures, which indicating a more packed structure and central bands for UM at 1688.07 cm^{-1} indicates more β-turn structures followed by CWS gel prepared from surimi possess central bands at 1653.20 cm^{-1}. The disappearance β-helix structure desired the formation of more β-sheet and β-turn as an ordered network due to heating of protein structures which is unfolded. Partial unfolding of a secondary structure for UM during solubilisation process leads to high band intensity having more random/loop arrangements (Zhou *et al.,* 2014). The β-sheet in CWS gels prepared from CWS, exhibit proper gelation after setting which coincides with the ideal gel strength (Table 2).

Microstructure of UM, CWS and CWS gel

The SEM micrographs of the surface layers of the thermal gels at 10000 × magnification are shown in Fig 3. The UM shows slack and grainy surface with various size intervals and imprecise network structure. Pores of various sizes in UM might due to the lower pH formed which leads the myosin

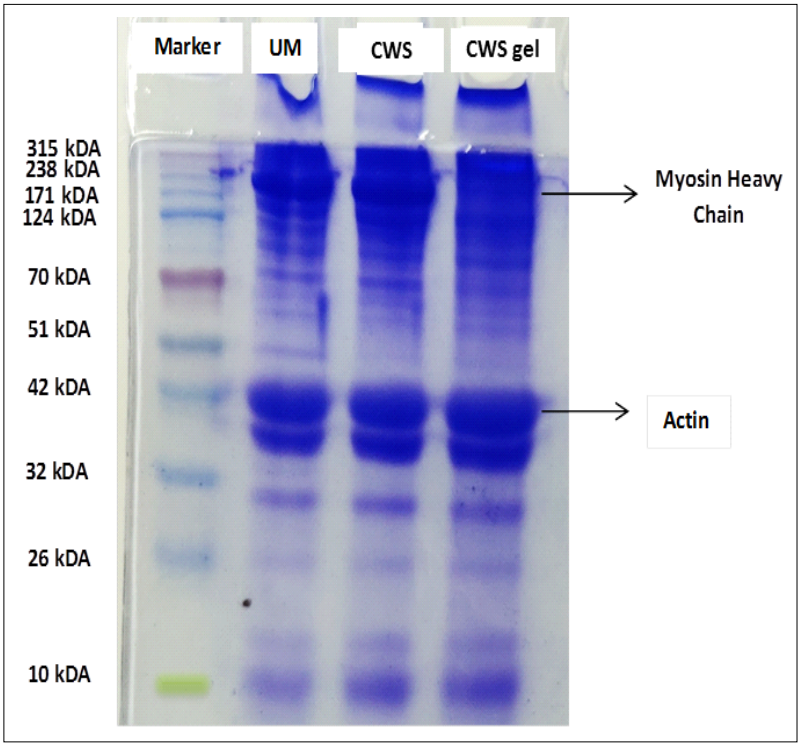

Fig 2: Protein pattern of UM, CWS and CWS gel.
UM- Unwashed mince, CWS- Conventional washed surimi.

to form a coarse and disordered gel network (Liu *et al*., 2010). The firmness and devoid of gaps in CWS might be attributed due to hydrophobic interactions which forms a three dimensional network structure with the reactive group of solubilized surimi protein (Weng and Zheng, 2015). In case of CWS gel, more particulate structure was observed and this might be due salt present in the gel sample which is beneficial to soften and swell the myofibrillar proteins, fostering protein-protein cross-linking leads to uniform and denser network (Kang *et al*., 2015).

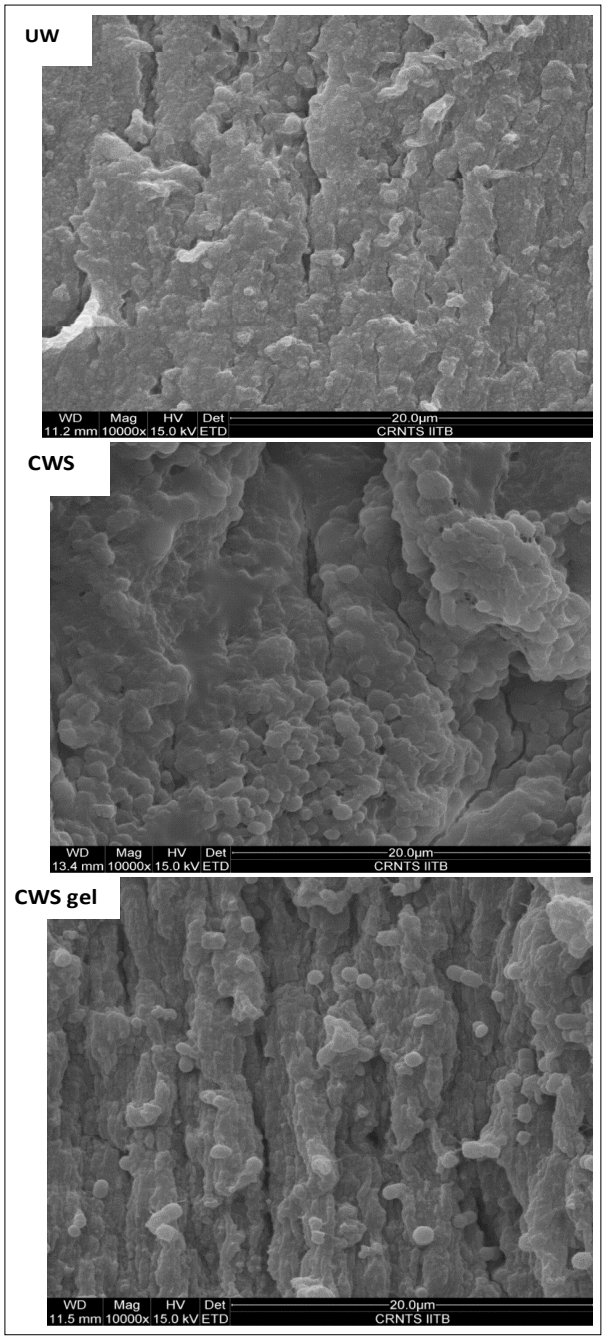

Fig 3: Microstructure of UM, CWS and CWS gel
UM- Unwashed mince, CWS- Conventional washed surimi.

CONCLUSION

Mince (UM), surimi (CWS) and CWS gel obtained from pony fish (*Leiognathus* sp.) were subjected to spectral and textural analysis and the values obtained suggesting the potential use of this low cost fish in surimi industries. However, certain quality attributes of gels can further be improved by suitable additives there by increasing the gel strength, water holding capacity, colour and other textural parameters in order to fulfill the requirements of commercial grade (A, AA, FA, SA) surimi. Microstructure analysis from the present study gives more precise image about internal structure and changes in fish muscle pattern for different treatments. From this study it is evident that low cost pony fish (*Leiognathus* sp.) showing good textural attributes which can be used an alternative source for major fish species used in surimi industries thereby minimizing the fishing pressure on other commercial species, reduce the cost of production and supply functional protein based product to the consumer at cheaper cost.

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Conflict of interest: None.

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