ENHANCEMENT OF GEL STRENGTH OF CROAKER SURIMI USING EXTRACTS OF BROWN SEAWEED (*Padina tetrastomatica*) AND GREEN SEAWEED (*Caulerpa sp.*)

POOJA SIDDHARTH GAMARE
B.F.Sc.

Department of Fish Processing Technology and Microbiology
College of Fisheries, Shirgaon, Ratnagiri - 415629
Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli
(Maharashtra State, India)

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THESIS

Submitted to the

Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli

In partial fulfillment of requirements for the degree of

MASTER OF FISHERIES SCIENCE

IN

FISH PROCESSING TECHNOLOGY

BY

POOJA SIDDHARTH GAMARE

B.F.Sc.

Under the guidance of

Shri. S. S. Sawant
Assistant Professor,
Department of Fish Processing Technology and Microbiology

College of Fisheries, Shirgaon, Ratnagiri – 415 629

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Approved by the Advisory Committee

Chairman and Research Guide

Shri. S. S. Sawant
Assistant Professor
Department of Fish Processing Technology and Microbiology
Members

Dr. S.B. Patange	**Dr. J.M. Koli**	**Dr. B.M. Yadav**
Professor, (CAS)	Professor, (CAS)	Assistant Professor
Department of F.PTM	Department of F.PTM	Department of F.RESE

Date:

Place: Ratnagiri

CANDIDATE'S DECLARATION

I hereby declare that the thesis entitled **"Enhancement of gel strength of croaker surimi using extracts of brown seaweed (*Padina tetrastomatica*) and green seaweed (*Caulerpa sp.*)"** is an authentic record of the research work done by me and that no part thereof has been submitted by me or any other person to any other University or Institute for a degree or diploma, associate-ship, fellowship or any other similar title.

Date:

Place: Ratnagiri

(Miss. Pooja Siddharth Gamare)

Reg No. FRRTM0170376

Dr. BALASAHEB SAWANT KONKAN KRISHI VIDYAPEETH, DAPOLI
COLLEGE OF FISHERIES, RATNAGIRI

(Certificate to be submitted by the supervisor of the candidate supplicating for M. F. Sc. degree along with thesis)

With regard to the thesis entitled **"Enhancement of gel strength of croaker surimi using extracts of Brown seaweed (*Padina tetrastomatica*) and Green seaweed (*Caulerpa sp.*)"** Submitted by **Miss. Pooja Siddharth Gamare** for the degree of this University.

I certify that:

1. She has carried out research work under my direct supervision and guidance during the academic year 2018-2019 and that the manuscript of the dissertation has been scrutinized by me.
2. The entire thesis comprises the candidate's own work and it is his own achievement. It has not previously formed the basis for the award of any degree, diploma, associate-ship, fellowship or other similar title of recognition.
3. The thesis does not contain any conjoint research work with me or anyone else.
4. She has completed his research work to my entire satisfaction.
5. The final typed copy of the thesis, which is being submitted to the University office, has been carefully read by me for its material and language and it is to my entire satisfaction.

Shri. S. S. Sawant
Assistant professor
Department of F. PTM

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(Miss. Pooja Siddharth Gamare)

Date

Place: Ratnagiri

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ACRONYMS

et al.	Et all (and others)
CE	Catechin
g	Gm
mPa.s	milipascal-second
GAE	Gallic acid equivalent
DS	Dry sample
WE	Water extract
EE	Ethanol extract
LE	Linoleic acid extract
TI	Trypsin inhibitor
SDS-PAGE	Sodium dodecyl sulphate - Polyacrylamide gel electrophoresis
MHC	Myosin heavy chain
WKWE	Water kiam wood extract
EKWE	Ethanol kiam wood extract
CT	Commercial tannin
OFA	oxidised ferulic acid
OTA	oxidised tannic acid
OCF	oxidised caffeic acid
OCT	oxidised catechin
WSE	Water seaweed extract
CWPS	Conventional washing process surimi
ASWPS	Alkaline-saline washing process
PPP	Porcine plasma protein
E60	Ethanol 60%

E80	Ethanol 80%
ANOVA	Analysis of Variances
Fig	Figure
h	hours
t	tonne
%	Percentage
ml	Millimetre
kg	kilogram
NSD	No significant difference
SD	Significant difference
SNK	Students Newman Kuels
^{o}C	Degree Celsius

ABSTRACT

Surimi is Japanese term which can be defined as concentrated myofibrillar protein obtained from fish flesh by washing to remove lipids, bloods, sarcoplasmic proteins and enzymes and mixed with cryoprotectants for frozen storage. Recently, plant phenolic compounds are used as protein cross-linkers. In present study, the brown seaweed (*Padina tetrastomatica)* and green seaweed (*Caulerpa peltata*) available along Ratnagiri coast of India were used. The total plant phenolic compound extracted from both brown and green seaweed as protein cross-linkers in croaker fish surimi. Water seaweed extract of *Padina tetrastomatica* (Brown) and *Caulerpa peltata* (Green) contained 16.75 and 15.34 mg tannin/g of dry seaweed powder. The effect of addition of different concentrations (0.0%, 0.2%, 0.4%, 0.6%, 0.8%)of seaweed on croaker surimi gel were studied. The 0.6% concentration of brown and green seaweed with surimi gel showed significantly increase in gel strength compared with control ($P<0.05$). The lower moisture content was showed in 0.6% concentration of surimi gel with both seaweeds concentration. However, addition of brown and green seaweed had slightly decrease in whiteness ($P<0.05$). During study low protein solubility (%) was found that, at 0.6% concentration of both seaweeds extracts. The addition of brown and green seaweed extract showed the minor increase in pH with increasing concentrations, which was not significant difference compared with control surimi gel sample ($P>0.05$). Folding test indicates that, the high score was found in 0.6% concentration of surimi gel with both seaweed compare to control. Therefore, it may be concluded that, the brown and green seaweed extract used as gel enhancer in croaker surimi has no changes in texture and flavour.

1.0 INTRODUCTION

The marine fish landings from the coast of the main land of India in 2017 was estimated as 3.83 million tonnes (t) showing an increase by 5.6% compared to the landings in 2016. The landing of croakers was 1.50 lakh tonnes (CMFRI, 2018). Surimi a Japanese word refers to a paste made from fish meat. It is refined fish myofibrillar proteins produced through various step-by-step processes including heading, gutting, filleting, deboning, and washing, dewatering, refining, mixing with cryoprotectants and freezing (Park, 2000).India's first surimi plant was set up in 1994.In India, generally lean fishes are used for the surimi production mainly Bigeye snapper (*Pricanthus spp.)*, Threadfin bream (*Nemipterus spp*), Croaker (*Pennahia and Johnius spp*). Croakers (*Shiroguchi* in Japanese) gives a low-grade surimi (low gel, darker colour). It is processed as a single species in India and is used in mixed fish surimi in Vietnam and China (Park,2000). The fatty fish are used for the surimi production because of the over exploitation of lean fishes.

Depending on the desired texture and flavour of the surimi product, the gelatinous paste is mixed withdiffering proportions of additives in the mince such as the starch, egg white, salt, vegetable oil, humecants, sorbitol, sugar, soya protein, seasoning and enhancers such as transglutaminase and Monosodium glutamate (MSG). The meat paste is mixed with food grade cryoprotectants which are used as preservatives when the surimi is utilised for packaging and being frozen.

In Southest-Asia, Thailand is the largest surimi and crabstick producing country with both volume and technology (Park, 2000). About 16 surimi factories are located in Thailand, with a total production of 96,500 to 1,13,500 metric tons per year of which, 80% is exported to Japan, Korea and the remainder to Singapore and other countries (Hong and Eong, 2005). In India the surimi concept introduced in early 1990s when

Gadre set up the first surimi plant. Recent, There are now eight surimi plants working in India (Satam, 2002). In Maharashtra, Ratnagiri 1995 started Gadre Marine Exports surimi operations with technical assistance from Daerim (South Korea). Naik Ice and Cold storage put up a surimi line in Ratnagiri. During the same year, Amar Cold Storage in Porbandar started a surimi line using Korean machinery (Park, 2000).

The less gel property, due to lipid and myoglobin content were higher in dark muscle than in ordinary muscle of both sardine and mackerel *spp*. (Chaijan et al., 2004). The textural properties developed during surimi 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., 2004b). Various food ingredients and protein additives have been used in surimi industry to improve the gel strength of surimi gel. Therefore, the addition of ingredients causes adverse effect on flavour and colour of surimi gel (Rawdkuen and Benjakul, 2008). As, it was reported by Balange and Benjakul (2009a) that addition of bovine plasma protein leads to mad cow disease, while egg white create allergy problems. Use of microbial transglutaminase (MTGase) i.e. cross-linking enzyme is costly in surimi preparation. There is need to find solution for improving the gel strength of fish surimi hence, the natural additives are used for enhancing the fish surimi. The natural additives have antioxidant activity. These are derived from different parts of the plants like Kiam wood extract, which act as a natural additive containing the phenolic compound i.e. tannin, used for enhancing the gel strength (Balange and Benjakul, 2009c). Different oxidized phenolic compounds are used for enhancing gel strength of mackerel surimi. Since, they induce conformational changes and cross-linking through amino groups or the induction of disulfide bond formation. (Balange and Benjakul, 2009b). Another way to solve the overcoming problem in surimi industry is to find out the novel and natural additive capable of gel strengthening. Plant phenolic

compounds, also denoted polyphenol, are defined as compounds possessing one or more aromatic rings bearing hydroxyl substituents, which are derived from the secondary metabolism of plants (Robards et al., 1999; Parr and Bowell.,2000). Recently, especially in low quality fish use of phenolic compounds in appropriate form at different concentration was found to be used to enhance the surimi gel strength. Generally phenolic compounds are natural additive and can be derived from different parts of plants.

The marine algae commonly known as "Seaweed" is shrub like structure that are generally attached to rock or other hard substrata in coastal areas. Seaweeds are important source of bioactive natural substances. Seaweeds have valuable medicinal value compounds such as antibiotics, antioxidant (Lekameera et al., 2013). The Indian marine environments have been reported 770 species of seaweed (Sahoo, 2010). Brown seaweed (*Padina tetrastomatica*) and Green seaweed (*Caulerpa sp*.) is very common in the west coast of India. *Padina* sp- Plants bunch with perennial prostate richly branched basal portion forming a distinct holdfast and attached to substratum by means of tufted rhizoids on dead corals and also on rocky surface. Fronds erect, 12-15 cm height, brownish to olive green in colour, broadly fan-shaped at upper portions, blades frequently lobed and spilt into numerous segments of 1-2 cm broad. Margin is revolute and curved. Zonation caused by rows of hairs and fructification organs in concentric zones. Hairs present in younger thallus, in older ones, either rudimentary or absent. Fronds are thick and leathery in nature (Sahoo, 2010). *Caulerpa sp.* is characterized by the usually unbranched, narrow, feather-like erect branches. Branchlets flat, constricted at the base and sickle-shaped. It forms a source for production of sodium alginate, animal feed, fertilizer and medicine (Sahoo,2010). Seaweed contains phenol level up to 20% of their dry weight (Connan and Stengel, 2007). Tannin (Phenolic character) is

present in marine algae *Padina sp*. (Vimalabai et al.,2004). Pharmacologists, Physiologists and Chemists started to give more attention towards seaweed to marine organisms for bioactive compounds (Arunkumar et al.,2010). Polyphenolic compounds have several hydroxyl (OH) groups. They occurin foods of plant origin. Since, it was expected to express radicalscavenging effector sometimes to have pro-oxidant effect as a source of reactive oxygen species (Yoshie et al.,2002). In brown algae, phenolic compounds are present in considerable quantity (Ragan and Glombitza, 1986). This increase the value of seaweed and thus it helps to introduce a novel natural additive in food industry, especially in surimi industry. However, there is limited information on the utilization of seaweed extracts as the cross-linking agents in food proteins, particularly myofibrillar proteins.

The specific objective of present research was summarized below.

OBJECTIVE:

To study the effect of different concentrations of dry seaweeds extract as gel enhancement on the croaker surimi.

2.0 Review of literature

2.1 Extraction of phenolic compounds from dry seaweed

Zehra (2007) conducted experiment on water and ethanol extracts (WE and EE), prepared from the dried sample of brown algae (*Sargassum boveanum*) and examined for its phenolic compounds. The total phenolic content was about 17 ± 0.492 mg catechin equivalent (CE)/g of dry sample in water extract, using Folin-Ciocalteu method.

Zubia et al. (2008) determined the alginate properties, mannitol and phenolic contents, antioxidant and antimicrobial activities of two range-extending brown algae from Tahitian coral reefs, namely *Sargassum mangarevense* and *Turbinaria ornata*. The richest alginate content in *Turbinaria ornata* with the highest extraction yield i.e.19.2± 1.3% distilled water. The highest viscosity showed in their alginates i.e.50±18 mPa.s, and in both species the M : G ratio (Mannuronic acid to Glucuronic acid) of alginates (1.25–1.42) were similar.

Chandini et al. (2008) reported phenolic compounds are commonly found in plants and to have several biological activities including antioxidant activity. Aqueous fraction of *S. marginatum* and *T. conoides* exhibited higher phenolic content of 24.61 and 49.16 mg GAE/g of seaweed extract (or 0.29 and 0.86 mg GAE/g of seaweed on dry weight basis), respectively, as compared to other fractions and total methanolic extract. Aqueous fraction of *P. tetrastomatica* also showed higher content (20.04 mg GAE/g of extract or 0.61 mg GAE/g seaweed on dry weight basis) as compared to phenolic content of other fractions from the same species.

Hwang et al. (2010) observed that, *Sargassum hemiphyllum* contained 0.240 mg phenolic content/ml of freeze dried hot water extract.

2.2 Quantification of total phenolic content

Oki et al. (2002) studied to clarify the contribution of anthocyanin and phenolic compounds-rich fractions to the radial scavenging activity, extracted by using 80% ethanol extracts of 5 purple-fleshed sweet potato cultivars, separated into 2 fractions.

Kuda et al. (2005) studied the antioxidant properties of the dried brown algae (*Sargassum. lomentaria).* He tested total phenols by using water extract revealed that 5.5 mg catechin equivalents (CatE)/g dry sample (DS) and showed antioxidant activities in all 5 different testing. The total phenol contents in WE, EE and LE were 5.57, 0.42 and 4.73, respectively as mg CatE/g dry sample (DS). The phenolic content in WE was similar or higher than that of common algae, such as Laminaria, Undaria and Porphyra (Jimenez-Escrig et al., 2001).

Shitole et al. (2014) conducted research on the total phenolic content extracted with water for three different sizes of seaweed powder. It was observed that the extract prepared with 0.5 g dry seaweed contained 12.70 mg tannin/g of dry seaweed powder. The 1.0 g dry seaweed powder preparedextract contained 16.24 mg tannin/g of seaweed powder. The higher amount of phenolic content was found in the extract prepared with 1.5 g seaweed powder was 21.85 mg tannin/g of dry seaweed powder. From results, phenolic content was increased due to increasing quantity of seaweed powder. With the 1.5 g seaweed powder, the colour of the extract became dark as compared with 0.5 and 1.0 g seaweed powder extract. Therefore,in the extraction of phenolic compound it was decided to use 1.0 g seaweed powder as standard sample size and for further use in determining quality of surimi.

2.3 Surimi:

Chaijan et al. (2004) characterized dark and ordinary muscle from sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*). Lipid and myoglobin contents were higher in dark muscle than in ordinary muscle of both species, and higher contents of both constituents were found in sardine muscle than mackerel muscle. The extractable myoglobin contents in sardines' dark and ordinary muscle were 14.27 and 2.18 mg/g, while mackerel dark and ordinary muscle contained 4.88 and 1.37 mg myoglobin/g sample, respectively. Alkali-soluble protein and stroma contents were greater in dark muscle than ordinary muscle. The higher content of non-protein nitrogenous compounds in mackerel muscle than sardine muscle. The effect of washing conditions on the myoglobin extractability was investigated. A large amount of myoglobin was removed in the first washing cycle and only a small amount was removed in the second washing cycle. The highest removal of myoglobin from sardine (32.10–46.55%) and from mackerel muscle (103.20–313.66%) was achieved when the mince was washed with 0.2% NaCl and 0.5% NaCl, respectively. Washing media showed the marked effect on the colour, expressible drip and textural properties of sardine and mackerel mince gels. The breaking force of directly heated and kamaboko gels from both sardine and mackerel mince washed with NaCl solution was higher than that of unwashed mince and water washed mince.

Kristinsson et al. (2005) prepared surimi of catfish muscle by conventional method. The freezing step was excluded and cryoprotectants were not added. Ground catfish muscle mixed into 3 volumes of cold water (4^{o} C) and stirred with rubber spatula for 15 min, following a 15 min period of settling. Then, slurry was dewatered by pouring it into a strainer lined with two layers of cheesecloth followed by squeezing loosely bound water out of the washed material. This process was repeated 2 times,

with the last wash including 0.2% NaCl to aid in dewatering. All steps were performed on ice.

Kristinsson and Liang (2006) prepared croaker *(Micropogonias undulates)* surimi by conventional laboratory process. Ground croaker muscle (1 part) was mixed with 4 parts cold (4^o C) deionized water and allowed to sit for 15 min after 3 min stirring. The washed croaker was then strained using 3 layers of cheesecloth lined in a strainer. The washing and straining process was repeated 2 more time, with the last wash including 0.2% NaCl in the deionized water.

2.4 Surimi gel

Benjakul et al. (2001) studied gel properties of Bigeye snapper surimi as affected by setting temperature, time and addition of porcine plasma proteins. Breaking force and deformation of suwari gels increased when surimi sol containing 0.5% PPP was incubated at higher temperature ($p<0.05$). No significant differences in both breaking force deformation were observed between suwari in presence of 0.5% PPP was in the range of 35-40^{o}C,in which breaking force and deformation approached the maximum.The decrease in solubility of the resultant suwari and kamaboko gels in solution containing sodium dodecyl sulfate, urea and 8-mercaptoethanol.

Klomkloa and Benjakul(2015) studied trypsin inhibitor (TI) from adzuki bean seed was partially purified by heat-treatment at 90^0C for 10 min and it was used to study the impact on proteolysis and gelling properties of threadfin bream (*Nemipterus bleekeri*). TI showed the inhibitory activity against sarcoplasmic proteinases and autolysis of threadfin bream mince and washed mince in a concentration dependent manner. TI was effective in proteolysis prevention as shown by more retained myosin heavy chain (MHC) on SDS-PAGE. Effect of TI (0.5, 1, 2 and 3 g/100 g) on the properties of threadfin bream surimi was also investigated.

Cando et al. (2016) tested the effect of adding tetra-sodium pyrophosphate, cystine and lysine as surimi gelation enhancers (Alaska Pollock) in order to reduce the sodium content of gels up to 0.3%. These gels were compared with others that contained 3% NaCl content (the amount typically used for surimi processing). To induce protein gelation, gels were first heated and then set at 5^0C/24 h. Once the physicochemical and rheological properties of the gels were determined, cystine and lysine were found to be the most effective additives improving the characteristics of low NaCl surimi gels. The action of these additives is mainly based on the induction of myofibrillar protein unfolding thus facilitating the formation of the types of bonds needed to establish an appropriate network. It was found that a setting period needed for gel processing to maximize the effect of the additives.

2.4.1. Estimation of gel strength:

Balange and Benjakul (2009b) conducted experiment on Kiam (*Cotylelobium lanceotatum craih*) wood. Water kiam wood extract (WKWE) and ethanolic kiam wood extract (EKWE) contained 251.90 and 456.30 mg tannin per g of dry extract, respectively. Effects of WKWE and EKWE at different levels (0–0.60% of protein content) on the properties of gels from mackerel (*Rastrelliger kanagurta*) surimi were investigated in comparison with commercial tannin (CT). Gels added with 0.30% WKWE, 0.15% EKWE or 0.30% CT had the increases in breaking force by 134.81%, 136.09% and 121.34% and in deformation by 52.60%, 54.96% and 33.53%, respectively, compared with the control (without addition of extracts or CT).

Balange and Benjakul (2009) determined the effect of different oxidized phenolic compounds at different protein content levels on the gel property of mackerel surimi. Gels added with 0.40% OFA, 0.50% OTA, 0.50% OCF or 0.10% OCT had the increases in breaking force (gel strength) by 45%, 115%, 46.1% and 70.3% and in

deformation by 12.2, 27.5, 28.1 and 28.4%, respectively, compared with the control (without addition of oxidized phenolic).

Shitole et al. (2014a) studied lesser sardine surimi gels were prepared from 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 % seaweed extract concentrationcontaining with total phenolic compound of 16.24 mg tannin/g of dry seaweed powder. It wasobserved that there was increase in gel strength with the increase in concentration of the WSE up to aparticular level. An experiment was conducted to see the effect of overnight frozen storage on the surimi samples kept without and with cryoprotectants at -20^{o}C. All the surimi gels prepared with WSE and without addition of any cryoprotectants and stored at -20^{o}C have good gel formation compared with gel prepared with cryoprotectants and without any WSE. The overnight storage at without any cryoprotectants might not have affected the gel-formation ability of myofibrillar proteins. Surimi gel with 2 %WSE had the significant ($P<0.05$) increase in gel strength up to 104 g/cm, compared with that of the controlgel, i.e. 51 g/cm. For gels added with 0.5 % of WSE, the minor increase in gel strength up to 97 g/cm was observed compared with that of the control gel, i.e. 51 g/cm. Progressive increase in gel strength was observed when WSE concentrations were used from 0.5 to 2.0 %. With 2.5 % WSE concentration, gel strengthdecreased, but the gel strength was higher than that of control. Use of WSE at 2 % concentration, increased the lesser sardine surimi gel strength by 76.27 %; compared to that of control surimi gel. Lesser sardine surimi gel prepared with addition of 2 % WSE showed significant increase in gel strength by 76.27 % as compared with control.

Solonki et al. (2011) determined the effect of egg albumen (protein additive) on surimi prepared from lizardfish (*Saurida tumbil*) during frozen storage. The biochemical, gel strength and sensory parameters were analyzed to study the quality changes and shelf life of these products in frozen storage at -20^{o}C. The addition of 3%

egg albumen exhibited gel enhancing effect by increase in gel strength 113.56 g/cm, whereas same treatment after 120th days of storage, total protein content was found to be higher i. e. about 12.69%, with compare to without egg albumen surimi.

Shah et al. (2018) has reported that the effects of egg white powder (EWP) and sodium ascorbate (SA); both individually and in combination at different levels on the gelation from surimi of lesser sardine (*Sardinella spp.*) were analysed. The addition of EWP and SA affected both the gelation and colour characteristics of surimi. EWP was added at concentrations of 0.5%, 1%, 1.5% and SA at concentrations of 0.1%, 0.2% and 0.3% and in combinations: 0.5% EWP with 0.1 and 0.2% SA and 1% EWP with 0.1 and 0.2% SA. The addition of EWP significantly increased the gel strength (*P* value >0.05) over the values of samples without EWP. Addition of SA at concentrations of 0.1%, 0.2% and 0.3% showed noticeably lesser values of gel strength than EWP.

2.4.2 Whiteness

Kristinsson and Liang (2006) observed the colour (L*, a*, b* values) of the surimi and protein isolates before and after gelation was measured. Both acid and alkali-aided isolates had significantly higher lightness (P<0.05) than surimi for both fresh and frozen cryoprotected samples. The lightness did increase (P<0.05) for the surimi on cooking, while the increase was much less for the isolates, hence resulting in a slightly higher L* value for the croaker surimi compared to the croaker isolates.

Chaijan et al. (2010) reported the highest whiteness in surimi gel prepared by conventional washing method, especially with 2-step heating (P<0.05). Due to the oxidation of myoglobin in the recovered proteins, the lower whiteness of gels of protein isolates with and without prewashing was possible. The conventional surimi gels exhibited a higher whiteness than those of the alkaline-aided protein (Perez-Mateos and Lanier, 2006).

Shitole et al.(2014) investigated decrease in whiteness of lesser sardine surimi gels was observed as the levels of the water seaweed extract (WSE) were increased. It was cleared that, with increasing concentration of WSE the whiteness decreased. It was 44.60^{o} when compared with control of 47.81^{o}. There was slight difference among treatment effect on whiteness of surimi gel ($P<0.05$).

2.4.3 Expressible moisture content

Kristinsson and Liang (2006) reported that the press losses of gels made from surimi, acid- aided isolates, and alkali-aided isolates directly after production were 8.25%, 6.96%, and 10.51%, respectively. The press losses of the gels made from cryoprotected frozen surimi, acid-aided isolates, and alkali-aided isolates were 9.68%, 7.52% and 6.17%, respectively. The higher moisture content in croaker gels than the chicken isolates. the press loss reading do not seem to be influenced by moisture content; that is the press loss was not lower for the cryoprotected frozen samples, except for the alkali-aided isolates.

Rawdkuen et al. (2009) studied the expressible moisture of kamaboko gels made from minced tilapia processed with conventional method, acid and alkaline aided processes were 5.39%, 7.85% and 5.90%, respectively. The lowest expressible moisture was found in the gel made from muscle protein washed by the conventional method. The result indicated that the protein network of that gel was higher in water-holding properties.In general, lower expressible moisture was coincidental with the increased breaking force. High expressible moisture was found in both kamaboko and modori gels prepared with acid and alkaline aided processes. This was possibly due to the poor gel network of pH-shifted mince. Therefore, gel matrices that could not imbibe water, led to high water releases. In addition, adjusting the pH of protein isolate to neutral can

enhance the unfolding of proteins to some extent and alter the water-binding property of protein.

Benjakul et al. (2008) tested expressible moisture content of gel added with different levels of MTGase and setting conditions. The increases in expressible moisture content were observed with increasing MTGase levels for gels, set at 25^{o}C for 2 h ($P<0.05$). For gels set at 40^{o}C for 30 min. prior to heating, the increases in expressible moisture content were noticeable as MTGase was added up to 0.4 units/g sample ($P<0.05$) and no changes were found with MTGase ranges of 0.4 to 0.8 units/g sample ($P>0.05$). After the appropriate setting, heating is required to induce the aggregation of protein, in which gel matrix formed can imbibe thewater. Increases in expressible moisture content were noticeable in gels with the addition of MTGase for both setting conditions.

Shitole et al. (2014) investigated the lowest expressible moisture content in lesser sardine surimi, when WSE at optimum level (2 %) was added. In prepared surimi gel (without WSE) increase in expressible moisture content was noted. The expressible moisture content was high in surimi prepared with WSE above optimum level. Among the WSE, the extract added at 2 % in surimi yielded the gel with the lowest expressible moisture content ($P<0.05$). At the optimal level, the cross-linking of proteins in the lesser sardine surimi gels could be enhanced. This 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.

2.4.4 pH

Shitole and Balange (2014) studied addition of water seaweed extract which showed insignificant increase in pH ($P<0.05$) with increasing concentration in surimi. The water seaweed extract added in surimi contain higher amount of phenolic compounds viz., polypenols, possessing one or more aromatic rings bearing hydroxyl substituent. The very less WSE quantity added in surimi, hence insignificant increase in pH was found.

2.4.5 Folding test

Kudo et al. (1973) studied the folding test, which was made by folding in half a slice of the "Kamaboko" product, 3 mm thick by 3 cm in diameter. If no cracking occurred along the fold, the slice was folded again perpendicular to the first fold. The 5- point rating scale of the folding test was based on the grades.

Kristinsson and Liang (2006) studied a folding test, where gel slice of 3 mm thickness (19 mm diameter) was cut and folded by hand at room temperature. The quality of the gels was determined according to its ability to fold once or twice using a 5-point system. Gels that pass a double fold without cracking are graded as score 5, the most elastic gels; gels that pass a single fold without cracking are graded as score 4; gels that crack gradually on a single fold are graded as score 3; gels that break immediately on a single fold are graded as score 2; and gels that break under finger pressure are graded as score 1. Duplicate fold tests were performed on each gel produced. All of the gels made from surimi, acid-aided isolates, and alkali-aided isolates, regardless of the absence or presence of cryoprotectants, passed a double-folding test without cracking, getting the best score of 5.

Shaviklo (2006) conducted the folding test by folding a 5 mm thick slice of gel slowly in half and in half again while examining it for signs of structural failure

(cracks). Three or more slice piece of the same inspection sample were folded completely in half for 5 seconds and changes in the shape were evaluated using five stage merit marks. The average values for three trials were calculated.

2.4.6 Protein solubility

Chawla et al. (1996) reported solubility studies in different solvents were compared. Washed mince had a salt (0.6M KCl)-soluble protein content of about 50%, but it was reduced to 3% after gelation by acetic acid, and further reduced to 2% after heat treatment. The solubility in 20 mM Tris-HCl buffer (S2), pH 8.0, was,10% for all samples. Addition of 1% SDS(S3), 8M urea and 2% β-mercaptoethanol gave solubilities 90%. However, for unheated and heated acetic acid-induced gels the solubility values in solvents 20 mM Tris-HCl buffer (pH 8.0) containing 1% (w/v) SDS (S3), 20 mM Tris-HCl buffer (pH 8.0) containing 1% (w/v) SDS and 8M urea (S4)and 20 mM Tris-HCl buffer (pH 8.0) containing 1% (w/v) SDS, 8M urea and 2% (v/v) b-mercaptoethanol (S5) were 16, 56 and 82% and 14, 65 and 87%, respectively.

Benjakul et al. (2001) studied effects of setting temperature, time, and addition of porcine plasma protein (PPP) on gel properties of surimi from big eye snapper (*Priacanthus tayenus*).Solubility of suwari and kamaboko gels prepared under different setting times. Very low solubility of both suwari and kamaboko gels was observed in 0.6 M KCl (Sl) and 20 mM Tris-HC1 (S2). Lower solubility was obtained when the setting time increased, indicating more cross-linking formed. The lower solubility was observed in the samples pre-incubated for a longer time. When the solvent containing urea, SDS and and ME was used, higher solubility was observed in all samples. Therefore, it can be concluded that higher gel strength of surimi containing 0.5% PPP can be obtained asthe setting time increases up to 90 min at 35^{o} C.

Shitole et al. (2014a) studied solubility of surimi gels added with different concentrations of WSE. Solubility was found to be low in 2 % WSE of prepared surimi gel. However, all the gel samples prepared with WSE have low solubility as compared with control gel. The formation of protein aggregates during setting and heating the solubility were decreases in the gels with and without WSE. The lower solubility obtained in the present investigation is very well correlating with higher gel strength and lower expressible moisture when added with 2 % WSE in surimi gel.

2.4.7 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of gel -

Balange and Benjakul (2009a) studied protein patterns of gels from unwashed mince, conventional washing process surimi (CWPS) and alkaline-saline washing process surimi (ASWPS) without and with addition of oxidised tannic acid (OTA) at the optimum level yielding the highest breaking force and deformation. For unwashed mince gel, that containing 0.5% OTA, this had non-significant increase in both breaking force and deformation, was also determined for protein pattern.Myosin heavy chain (MHC) completely disappeared in the control gel samples (without OTA addition). Actin was found to be the dominant protein in the gel. For gels of CWPS and ASWPS added with optimal OTA level, a large amount of polymerised proteins as appeared on stacking gel. It indicated that non-disulphide covalent bonds were formed to a higher extent when OTA was incorporated. Protein cross-links might be more resistant to proteolysis caused by indigenous proteases.

Shitole et al. (2014) reported the protein patterns of surimi gels without and with the addition of water seaweed extract. For gels with addition of 2.0% WSE, the MHC band disappeared almost completely. No marked changes in actin band intensity were observed between the control gel and those with addition of different concentrations of

WSE. It was postulated that phenolic compounds might partially lower the proteolysis caused by endogenous proteinases.

2.5 Phenolic compounds

Phenolic compounds or phenolic substances that possess an aromatic ring with at least one hydroxyl group. They are the most widely distributed secondary metabolites and universally present in the plant kingdom (Cheynier et al., 2013).Phenolic compounds are major sources of these compounds in the human diet present in almost all foods of plant origin, fruits, vegetables, and beverages (Hertog, Hollman, Katan, and Kromhout., 1993).

Balasundram et al. (2006) studied the antioxidant activity of phenolic compounds depends on the positions of the hydroxyl groups, structure, in particular the number and the nature of substitutions on the aromatic rings.In plants, phenolic compounds are essential part of the human diet, due to their antioxidant role. In the human diet, beverages such as fruit juices, tea and wines are important sources of phenolics. The pomegranate peels contain 249.4 mg/g phenolics compared to 24.4 mg/g phenolics in the pulp (Li et al., 2006).

Balange and Benjakul (2009b) estimated effects of different oxidised phenolic compounds (ferulic acid, OFA; tannic acid, OTA; catechin, OCT and caffeic acid, OCF) at different levels (0–0.60% of protein content) on the properties of gels from mackerel (*Rastrelliger kanagurta*) surimi. Gels with addition of 0.40% OFA, 0.50% OTA, 0.50% OCF or 0.10% OCT had increases in breaking force by 45%, 115%, 46.1% and 70.3% and in deformation by 12.2, 27.5, 28.1 and 28.4%, respectively, compared with the control (without addition of oxidised phenolics).

Balange et al. (2009c) studied different composition of kiam wood extracts. The fine form showed the highest yield, among three different forms of kiam wood. The

total phenolic content of fine wood ethanolic and water extract had 498.44 and 198.99 mg tannin per g dry kiam wood extract, respectively. The ethanolic extracts contained higher total phenolic content than water extracts ($P < 0.05$). Water extract contained the lower tannin (251.90 mg tannin per g of dry kiam wood extract) than ethanolic extract (456.30 mg tannin per g of dry kiam wood extract).

2.6 Effect of different phenolic compounds on gel enhancement

Benjakul et al. (2001) studied effects of setting temperature, time, and addition of porcine plasma protein (PPP) on gel properties of surimi from big-eye snapper (*Priacanthus tayenus*). Breaking force and deformation of the surimi gels increased as the setting time and temperature increased. The gel pre-incubated at 35^{O}C for 90 min in the presence of 0.5% PPP, followed by cooking at 90^{o}C for 20 min showed the maximum force and deformation. The decrease in solubility of the resultant suwari and kamaboko gels in solution containing sodium dodecyl sulfate, urea and β-mercaptoethanol.

Shitole (2012) studied gel strength enhancement of Indian Mackerel (*Rastrelliger kanagurta*) surimi using seaweed extract. Phenolic compound used at different concentration i.e. 0.5%, 1.0%, 1.5%, 2.0%, and 2.5% were added in surimi, upto 32.45% gel strength increase was found within mackerel surimi, prepared with 2% dry seaweed extract, compared to control surimi. Whereas the use of fresh seaweed extract increased mackerel surimi gel strength upto 11.11%. Use of dry seaweed extract at optimum level i.e. 2%, along with oxidizing agent in mackerel surimi showed increase in gel strength by 31.88%. Therefore, it was concluded that the addition of dry and fresh seaweed extracts respectively at 2% and 0.5% improves the gel strength of mackerel surimi respectively. Additional improvement in gel strength of mackerel

surimi can be obtained with 2% dry seaweed extract along with 1% oxidizing agent H_2O_2.

Effects of coconut husk ethanolic extracts on gelling properties of surimi from sardine (*Sardinella albella*) were investigated by Buamard. N. And Benjakul(2015). Extracts prepared using 60% ethanol (E60) and 80% ethanol (E80) with total phenolic content of 464 and 454 mg tannin/g were incorporated into surimi gel. Gels added with E60 or E80 had the increase in breaking force as the levels increased and the highest breaking force was observed when added with 0.125% E60 and 0.075% E80 ($p<0.05$). Both E60 and E80 had no detrimental effect on sensory attributes of surimi gel.

3.0 MATERIAL AND METHODS

3.1 Materials:

3.1.1 Fish

Croaker fishes were purchased from Mikarwada jetty, Ratnagiri. The fishes were washed and cleaned.

3.1.2 Seaweed

Brown and green seaweed samples were collected, respectively from Aareware beach and Mirya creek of Ratnagiri coast.

3.1.3 Surimi

The fish mince obtained by using Deboning machine available with department of Fish Processing Technology and Microbiology, College of Fisheries, Ratnagiri.

3.1.4 Chemicals:

3.1.4.1 Tannic acid

Tannic acid powder was used as a standard for estimating phenol content from dry seaweed powder.

3.1.4.2 Folin-Ciocalteu phenol reagent and Sodium carbonate

Folin-Ciocalteu phenol reagent and sodium carbonate was used for the quantification of phenol content in seaweed extract by Spectrophotometric method.

3.1.4.3 Potassium chloride, Trichloroacetic acid and Sodium hydroxide

Potassium chloride, Trichloroacetic acid and Sodium hydroxide were used for the determination of protein solubility.

3.1.4.4 SDS Powder, Acrylamide/Bisacrylamide Solution, Ammonium persulphate (APS), Tetramethylethylenediamine (TEMED), Agarose, Staining and Destaining Solution:

These chemicals were used for the SDS-Polyacrylamide gel electrophoresis method.

3.1.5 Equipments and Machineries:

3.1.5.1 Electronic weighing balance

'Sartorius' (Citizen Scale Pvt. Ltd., Mumbai, India) make digital electronic weighing balance was used for the weighing purpose.

3.1.5.2 Deboning Machine

Barder 600 make Deboning machine was used for obtaining fish mince.

3.1.5.3 Autoclave

Equitron (Medical Instrument, Mumbai) make Autoclave was used for sterilization of seaweed extracts.

3.1.5.4 Centrifuge machine

Hettich (Mkro 220r) make centrifuge machine was used for centrifugation of seaweed extracts.

3.1.5.5 Spectrophotometer

Thermo spectronic (Genesys 10 UV) make spectrophotometer was used for the quantification of phenol content in seaweed extracts.

3.1.5.6 Waterbath

Bio Techno Lab make waterbath was used for preparation of surimi gel and SDS-PAGE.

3.1.5.7 pH meter

Equip-Tronics make pH meter was used for the estimation of pH.

3.1.5.8 Homogeniser

Remin Eletrotechnik Ltd. makehomogeniser was used for homogenized the sample.

Plate No.1. Deboning Machine

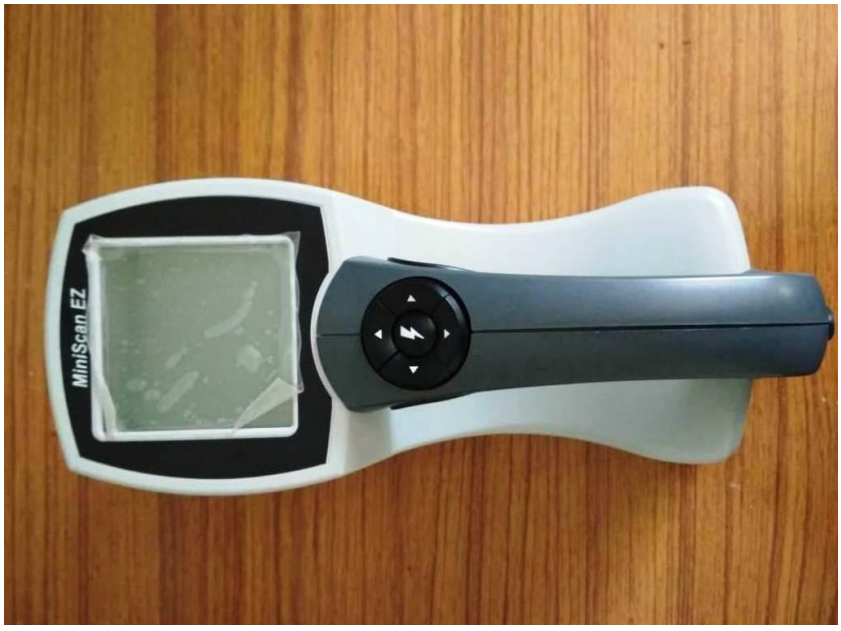

Plate No.2. Whiteness meter

3.1.5.9 Grinder

'Prestige' make mixer grinder was used for grinding the dry seaweeds.

3.1.5.10 Texture Analyzer

Perten (Texvol instruments TVT-300 XP) make texture analyzer was used for measuring gel strength.

3.1.5.11 Whiteness meter:

Hunter lab scan XE (Hunter Lab Model No 2500L) make whiteness meter was used for measuring the colour.

3.2 Methods:

3.2.1 Collection of seaweed along Ratnagiri coast of Maharashtra

Brown and green seaweed were collected by hand picking method and packed in polythene bags. Collected seaweeds were transported to the laboratory at College of Fisheries, Ratnagiri. After transportation, seaweeds were washed with fresh water.

3.2.2 Identification of seaweed

Identification of seaweeds according to CMFRI Bulletin no.20 by Rao (1970) , CMFRI Bulletin no. 41 by Silas (1987) and Sahoo D.,(2010).

3.2.3 Drying and packing of seaweed

Seaweeds were washed with fresh water and were kept for sun drying in solar tent dryer for 4-6 days. After drying, seaweed was grinded using a grinding machine. Then seaweed powder sieved using a test sieve of diameter 0.07 to 0.1 mm and available seaweed powder was packed in polythene bags and used as per requirement of research.

3.2.4 Standardization of extraction of phenolic compounds from dried brown and green seaweed

With slight modification in the method proposed by Zahra et al. (2007) seaweed extract was prepared for quantification of phenol content.

Plate No.3. Brown seaweed (*Padina tetrastomatica*)

Plate No.4 Green seaweed (*Caulerpa peltata*)

The seaweed powder - 0.5g, 1.0 g, and 1.5 gwas taken and dissolved in 100 ml of distilled water. Then it was labelled as A, B, and C. These samples were autoclaved at 121^0 C for 15 min. Autoclaved samples were centrifuge by centrifugation machine. After centrifugation, supernatant was removed by filtration and supernatant was evaporated directly on flame, to get final volume 10 ml. The final volume of seaweed extract was collected in glass bottles.

Preparation of dry seaweed extract is given in flow chart No. 3.1

Dry seaweed

⇩

Grinding

⇩

Sieving

⇩

Dissolve required quantity of seaweed powder in 100 ml of distilled water.

⇩

Autoclaved at 121^0C for 15 min

⇩

Centrifugation at 2000 rpm for 10 min

⇩

Supernatant was evaporated directly on flame, to get final volume 10 ml

⇩

Extract was collected in glass bottles

3.2.5 Quantification of total phenolic content

The quantification of total phenolic content was measured by the method given by Kuda et al. (2005) from different concentrations of seaweed extracts i.e. A, B and C. Then, 0.4 ml from each concentration i.e. A, B & C, of seaweed extract was transferred into separate test tubes. Then add 0.8 ml of the 10% Folin-Ciocalteu-phenol reagent in each test tube. After 3 min, 10 ml of the 10% sodium carbonate solution was added in each test tube. The test tubeswere mixed thoroughly and kept at room

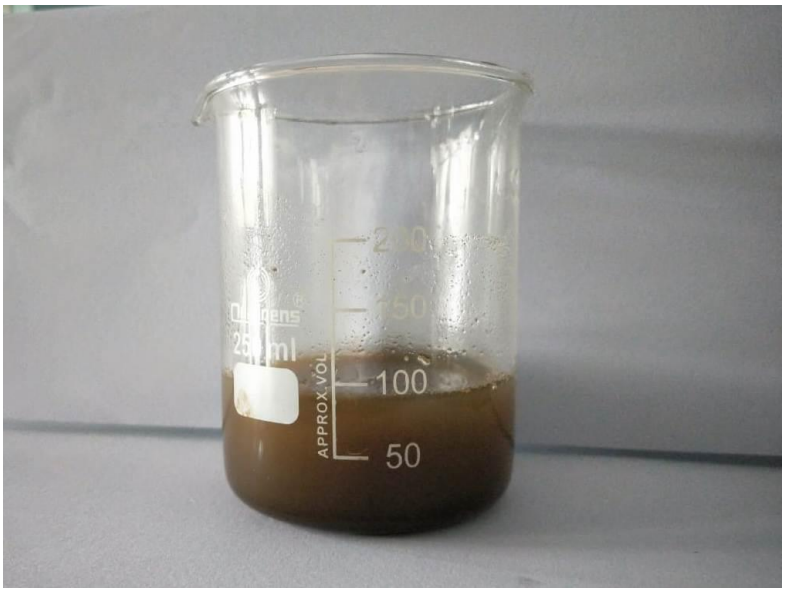

Plate No. 5. Brown seaweed (*Padina tetrastomatica*) extract

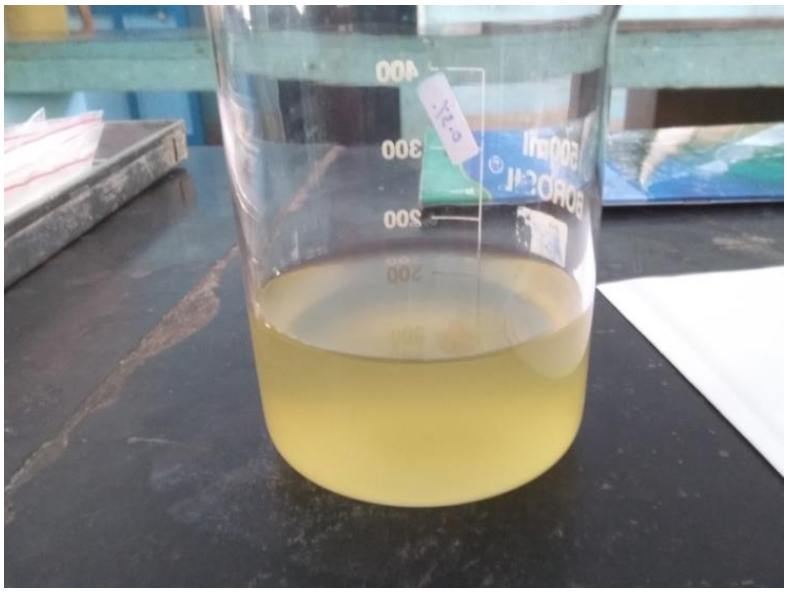

Plate No.6. Green seaweed (*Caulerpa peltata*) extract

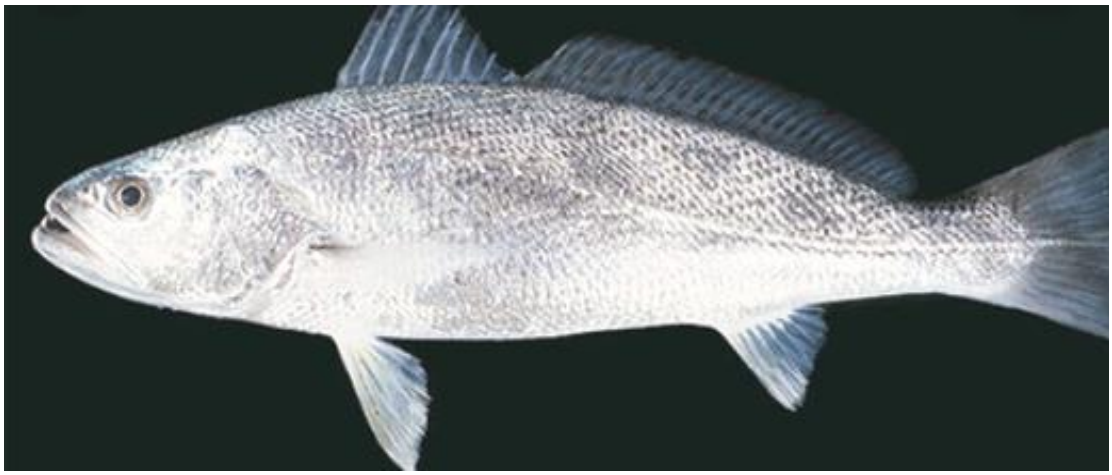

Plate No. 7 Tigertooth croaker (*Otolithes ruber*)

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Plate No. 8 Lesser tigertooth croaker (*Otolithes cuvieri*)

temperature for 1 hour in dark place. After that absorbency was recorded using a spectrophotometer at 750 nm in triplicates. Absorbency was measured for estimating the total phenolic compound from seaweed extracts i.e. from A, B & C against the standard curve of tannic acid and results were expressed in mg/g of dry seaweed extracts. Control sample was prepared by using 0.4 ml of distilled water instead of seaweed extract keeping other reagents and chemicals same as used for the samples.

3.2.6.Addition of seaweed extracts to surimi at appropriate levels and preparation of surimi gel

Croaker fish **(***Otolithus ruber***)** were purchased from Mikarwada jetty Ratnagiri. Surimi was prepared according to method given by Chaijan et al.(2004).Then surimi gel was prepared by following Balange and Benjakul (2009c) method. The phenolic compound content of brown seaweed (*Padina tetrastomatica*) extract was found to be 16.75 mg tannin/g of dry seaweed powder and the phenolic compound content of green seaweed (*Caulerpa peltata*) extract was observed to be 15.33 mg tannin/g of dry seaweed powder. Different concentrations of both types of seaweed extracts i.e. 0.0%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% was prepared and added in the surimi at low temperature. These prepared surimi samples were packed and stored overnight at -18 to -20^{o}C.

On next day, surimi was thawed at room temperature and it was chopped and 3% of saltadded in it. Then surimi sol (surimi added with different concentrations of seaweed extracts) and control sol (surimi without seaweed extract) was stuffed in krehlon casing and both ends of krehlon casing were sealed tightly. These casings were incubated at 40^{o} C for 30 min and 90^{o} C for 20 min in water bath. Such incubated casings were kept in ice for 20 min. for chilling purpose and stored overnight at 4^{o} C in an incubator.

3.2.7 Observation of gel strength characteristics of croaker surimi

3.2.7.1 Estimation of gel strength of surimi gel

The gel strength of different concentrations of surimi gel measured by texture analyzer. Surimi sausages were analysed at room temperature. These surimi sausages were cut into 2.5 cm height and breaking force (gel strength) and deformation (deformability/elasticity) was measured by texture analyzer. A probe having diameter of 5 mm and speed of 60 mm/min spherical plunger was pressed until puncture occurred into the cut surface of surimi sausage at a constant speed and distance both are recorded on texture analyzer (Balange and Benjakul, 2009c).

3.2.7.2 Determination of Whiteness

Whiteness of different concentrations of surimi gel was analysed by whiteness meter of Hunter lab scan XE (Hunter Lab Model No 2500L) using a method of Balange and Benjakul (2009c). The Whiteness was calculated using the value of L* (lightness), a* (redness/greenness) & b* (yellowness/blueness) by applying following equation (Park, 1994).

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

3.2.7.3 Determination of expressible moisture content

The expressible moisture content of surimi gel was measured according to method of Balange and Benjakul (2009c). The cylindrical surimi gel was cut into a thickness of 0.5 cm, weighed (X) and placed between three sheets of Whatman paper no.4 at the bottom and two sheets at the top, for 2 min by keeping standard weight of 5 kg on the top. Then sample was removed and weighed (Y). The expressible moisture content was measured by following equation:

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

3.2.7.4 Observations of chemical parameter

3.2.7.4.1 pH - The pH was measured using pH meter according to method of Balange and Benjakul (2009). 5 gm of surimi sausage sample was weighed and homogenized with 45 ml distilled water. Then this solution was filtered by using filter paper and the solution was placed for checking the pH with the help of digital pH meter.

3.2.7.5 Observation of physical parameters

3.2.7.5.1 Folding test

The folding test of gel sample was determined according to method of Shaviklo, (2006). The cylindrical surimi gel was cut into thickness of 5 mm. These slice gel folded into half and in half again until it gives signs of structural failure cracks. Same procedure was repeated for 5 seconds and changes were examined in the shape using five stage merit marks according to table No. 3.1

Table No.3.1 Folding test score of surimi gel

Sr. no.	Description of folds
5	If folded in four no crack was occurs
4	No crack occurs if folded in two but a crack(s) occur(s) if folded in four.
3	No crack occurs if folded in two but splits if folded in four.
2	Cracks if folded in two.
1	Splits into two if folded in two.

3.2.7.6 Solubility determination

The Protein solubility of surimi gel analysed by Benjakul et al. (2001). Different types of prepared surimi gel samples were taken in the quantity of 1 gm and homogenized with 0.6 M KCL for 1 min , boiled for 2 min and stirred the samples for 4 hour at room temperature (28-30^{0}c) using stirrer. The mixture was centrifuged at

6000×g for 30 min. After centrifugation, 10 ml of supernatant were added to 2 ml of 50% (w/v) cold Trichloroacetic acid (TCA). The mixtures were kept at 4^{o} C for 18 hour prior centrifugation at 10,000×g for 20 min and precipitate was washed with 10% (w/v) TCA, and solubilising in 0.5 M NaOH. The concentration of protein was determined by the Biuret method (Robinson and Hodgen, 1940).

3.2.7.7 Observation of protein patterns of surimi gel

3.2.7.7.1 SDS-PAGE (Sodium dodecyl sulphate - Polyacrylamide gel electrophoresis)

The SDS-PAGE (Sodium dodecyl sulphate - Polyacrylamide gel electrophoresis) was determined according to method of Laemmli, (1970). Preparation of protein sample, the 3 gm of surimi gel sample were added to 27 ml of 5% (w/v) SDS solution heated to 85^{o}C and homogenized at a speed 11,000 rpm for 2 min. Then incubated at 85^{o}C for 1 hour to dissolved total protein. After heating the samples were centrifuged at 3,500×g for 20 min**.** The supernatant of protein concentration was analysed by Biuret method (Robinson and Hodgen 1940) using standard of bovine serum albumin. The sample of gel mixed with sample buffer i.e. 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 g Bromophenol blue) at 1:1 ration (v/v). The 20 µg protein samples were loaded onto the Polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at constant current of 15 mA per gel using a Electrophoresis. 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.

3.2.7.8 Statistical analysis

The data was analysed by using the statistical methods of Snedecor and Cochran, (1967).

4.0 RESULTS

4.1 Collection of seaweeds along Ratnagiri coast of Maharashtra

Brown and green seaweed were collected and identified with help of CMFRI Bulletin No. 20 (Rao, 1970), CMFRI Bulletin No. 41 (Silas, 1987) and Sahoo (2010). The most available seaweeds along Ratnagiri coast were; *Sargassum tenerrimum, Chaetomorpha, Caulerpa taxifolia, Padina spp., Gelidium spp., Dictyota dichotoma,* and *Ulva fasciata*.

4.1.1 Identification of seaweeds

4.1.1.1 *Padina spp.*

Plants bunch with perennial prostate richly branched basal portion forming a distinct holdfast and attached to substratum by means of tufted rhizoids on dead corals and also on rocky surface. Fronds erect, 12-15 cm height, brownish to olive green in colour, broadly fan-shaped at upper portions, blades frequently lobed and spilt into numerous segments of 1-2 cm broad. Margin is revolute and curved. Zonation caused by rows of hairs and fructification organs in concentric zones. Hairs present in younger thallus, in older ones, either rudimentary or absent. Fronds are thick and leathery in nature (Sahoo, 2010).

4.1.1.2 *Caulerpa spp.*

Caulerpa sp. is characterized by the usually unbranched, narrow, feather-like erect branches. Branchlets flat, constricted at the base and sickle-shaped. It forms a source for production of sodium alginate, animal feed, fertilizer and medicine (Sahoo, 2010).

4.2 Calculation of Yield

4.2.1 Seaweeds

The fine form of seaweed dry powder (0.1 mm particle size) used for preparation of seaweed extracts. The 31.6g and 27.8g dry fine seaweeds powder were obtained from 1 kg of fresh brown (*Padina tetrastomatica*) and green (*Caulerpa peltata*) seaweed powders respectively.

4.2.2 Fish mince

Four hundred thirty gram of fish mince obtained from 1 kg of fish after deboning.

4.3. Standardization of extraction of phenolic compounds from dried brown seaweed (*Padina tetrastomatica*)

The total phenolic content extracted using different concentrations of brown seaweed (*Padina tetrastomatica*) obtained with concentrations 0.5, 1.0 and 1.5 were 13.98, 16.75 and 21.83 respectively. In present study, the concentration 1.0% was used for further experiment because the colour of surimi turning dark due to use of 1.5% seaweed concentration.

Table No. 4.1 Standardization of extraction of phenolic compounds from dried brown seaweed

Sample	Brown seaweed powder concentration (g)	mg tannin/g
A	0.5	13.98 ± 0.09
B	1.0	16.75 ± 0.01
C	1.5	21.83 ± 0.04

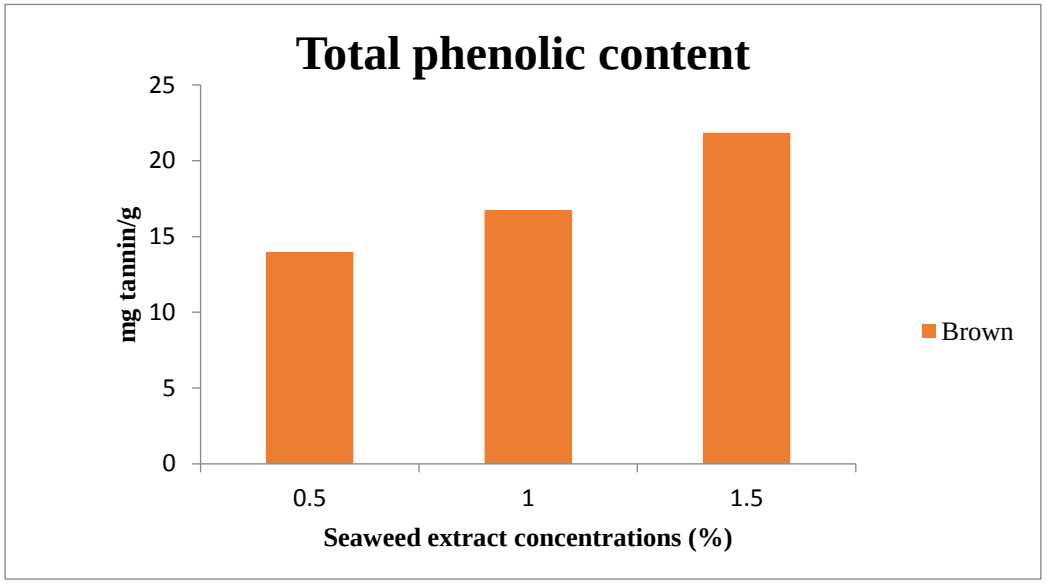

Fig. No. 4.1 Standardization of extraction of phenolic compounds from dried brown seaweed (*Padina tetrastomatica*)

4.4 Observation of gel strength characteristics of croaker surimi

4.4.1 Effect of dry brown seaweed (*Padina tetrastomatica*) extract on gel strength of surimi gel

The croaker surimi gels prepared with incorporation of different concentrations of seaweed extract are 0.0% (control), 0.2%, 0.4%, 0.6% and 0.8%. Allconcentrations of brown seaweed (*Padina tetrastomatica*) extract containing total phenolic compounds of 16.75 mg tannin/g of dry seaweed powder. As, 0.0% surimi gel gives 57 g/cm, 0.2% of surimi gel gives 61 g/cm, 0.4% surimi gel gives 68 g/cm, 0.6% surimi gel gives 73 g/cm and 0.8% of surimi gel gives 72 g/cm of gel strength respectively.

From the above observation it revealed that 0.6% seaweed extract added surimi gel gives better gel strength (73 g/cm) than the other concentrations including control surimi gel. Significant difference was observed between the control and 0.8% and 0.6 % concentrations of seaweed extract added surimi gel ($P<0.05$).

Table No. 4.2 Effect of dry brown seaweed (*Padina tetrastomatica*) extract on gel strength of surimi gel

Sample	Brown seaweed concentrations (%)	Gel strength (g/cm)
A	0.0	57 ± 1.00
B	0.2	61 ± 2.08
C	0.4	68 ± 3.06
D	0.6	73 ± 9.87
E	0.8	72 ± 7.55

4.4.2 Effect of dry brown seaweed (*Padina tetrastomatica*) extract on whiteness of surimi gel

The croaker surimi gels prepared with incorporation of different concentrations of seaweed extract are 0.0% (control), 0.2%, 0.4%, 0.6% and 0.8%. As, 0.0% surimi gel gives whiteness of 74.63°, 0.2% of surimi gel gives 72.86°, 0.4% surimi gel gives 71.94°, 0.6% surimi gel gives 70.83° and 0.8% of surimi gel gives 69.77° of whiteness respectively. The whiteness of surimi gels decreases with increasing seaweed concentrations. There was minor difference among treatments effect on whiteness of surimi gel ($P < 0.05$). Significant difference was observed between the concentrations 0.2% and 0.8% and between control and 0.4%, 0.6%and 0.8%.

Table No.4.3 Effect of dry brown seaweed (*Padina tetrastomatica*) extract on whiteness of surimi gel

Sample	Brown seaweed extracts concentrations (%)	Whiteness(°)
A	0.0	74.63 ± 0.79
B	0.2	72.86 ± 0.82
C	0.4	71.94 ± 1.65
D	0.6	70.83 ± 0.67
E	0.8	69.77 ± 0.66

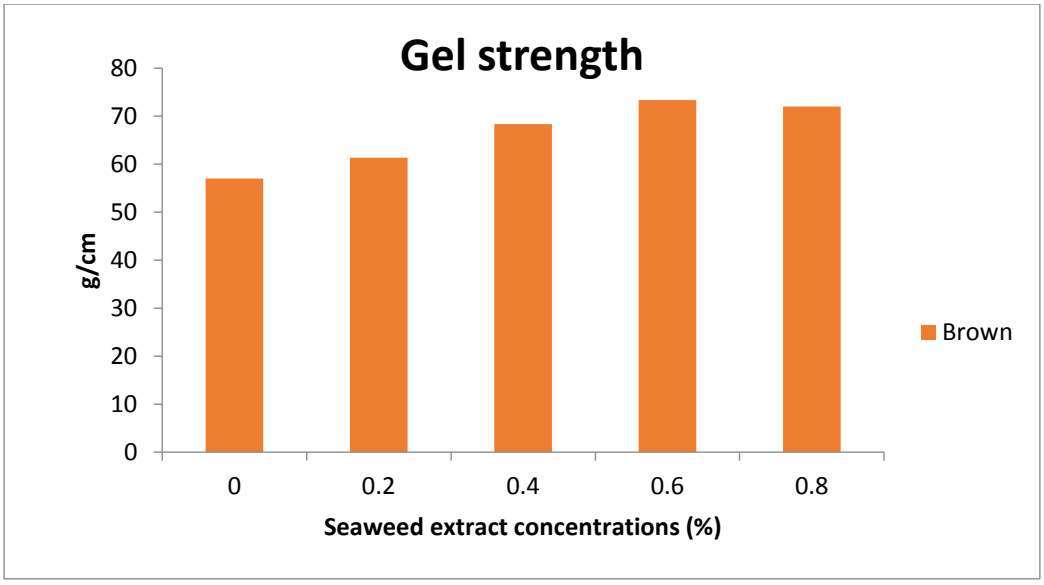

Fig. No. 4.2 Effect of dry brown seaweed (*Padina tetrastomatica*) extract on gel strength of surimi gel

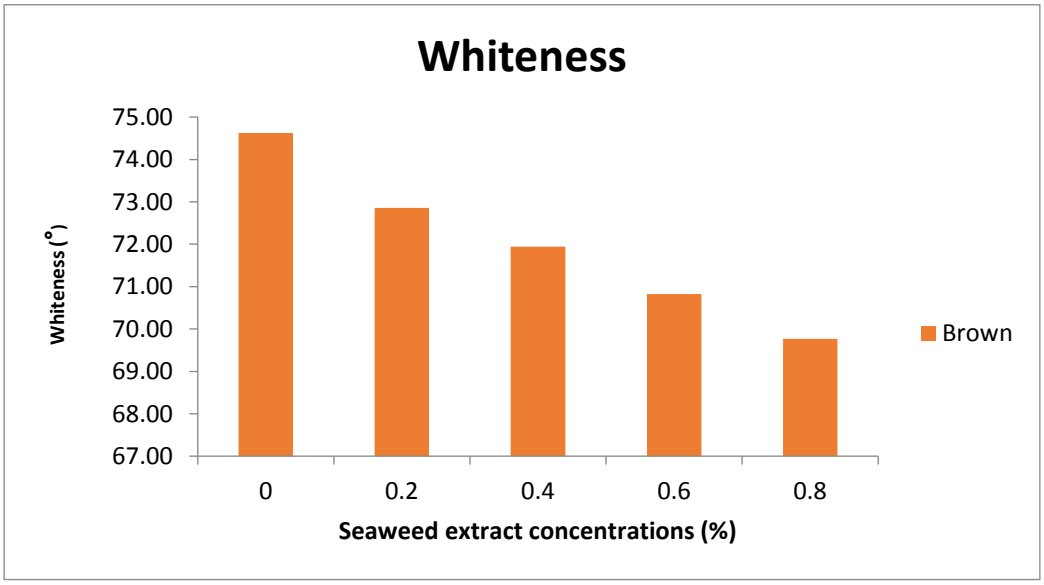

Fig. No. 4.3 Effect of dry brown seaweed (*Padina tetrastomatica*) extract on whiteness of surimi gel

4.4.3 Effect of dry brown seaweed (*Padina tetrastomatica*) extracts on expressible moisture content

The croaker surimi gels prepared with addition of different concentrations of seaweed extract are 0.0% (control), 0.2%, 0.4%, 0.6% and 0.8%. As, 0.0% surimi gel gives expressible moisture up to 4.47%, 0.2% of surimi gel gives 2.95%, 0.4% surimi gel gives 2.52%, 0.6% surimi gel gives 1.63% and 0.8% of surimi gel gives 2.35% of expressible moisture respectively. ANOVA indicates that,significant difference was observed between the concentrations of control and 0.6 % ($P < 0.05$).

Table No. 4.4 Effect of dry brown seaweed (*Padina tetrastomatica*) extracts on expressible moisture content

Sample	Brown seaweed concentrations (%)	Expressible moisture content (%)
A	0.0	4.47 ± 0.02
B	0.2	2.95 ± 4.47
C	0.4	2.52 ± 0.93
D	0.6	1.63 ± 1.34
E	0.8	2.35 ± 1.07

4.4.4 Effect of dry brown seaweed (*Padina tetrastomatica*) extracts on pH of croaker surimi gel

The croaker surimi gels prepared with incorporation of different concentrations of seaweed extract are 0.0% (control), 0.2%, 0.4%, 0.6% and 0.8% containing total phenolic compounds of 16.75 mg tannin/g of dry seaweed powder. As, 0.0% surimi gel gives pH 6.45, 0.2% of surimi gel gives 6.45, 0.4% surimi gel gives 6.46%, 0.6% surimi gel gives 6.47% and 0.8% of surimi gel gives 6.47% of pH respectively. The addition of brown seaweed (*Padina tetrastomatica*)extract containing phenolic compounds show the slight increase in pH with increasing concentrations, which was not significant compared with control surimi gel sample (P>0.05).

Table No. 4.5 Effect of dry brown seaweed (*Padina tetrastomatica*) extracts on pH of croaker surimi gel

Sample	Brown seaweed concentrations (%)	pH
A	0.0	6.45 ± 0.01
B	0.2	6.45 ± 0.02
C	0.4	6.46 ± 0.02
D	0.6	6.47 ± 0.02
E	0.8	6.47 ± 0.03

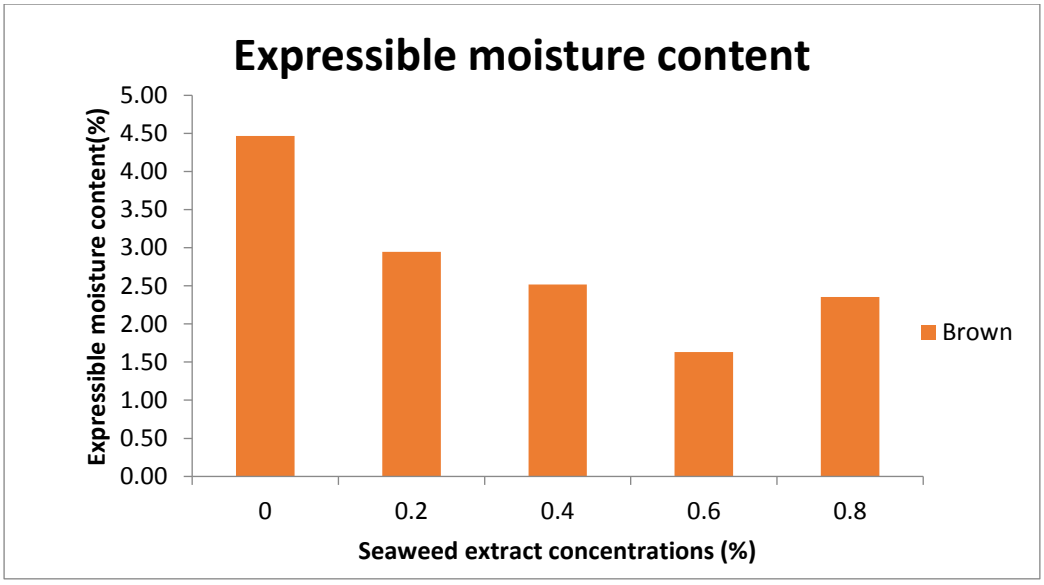

Fig.No.4.4 Effect of dry brown seaweed (*Padina tetrastomatica*) extracts on expressible moisture content.

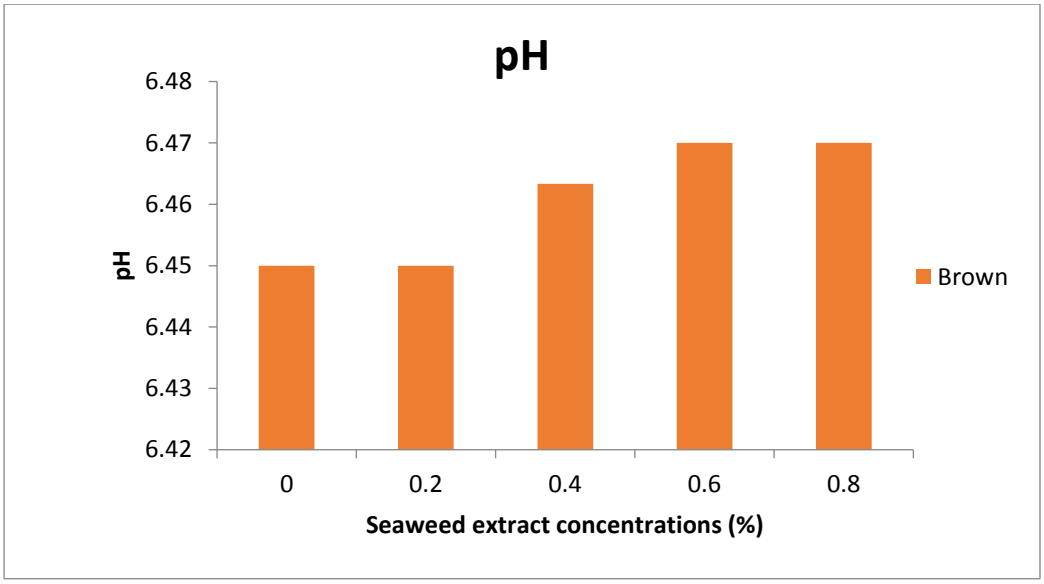

Fig. No. 4.5 Effect of dry brown seaweed (*Padina tetrastomatica*) extracts on pH of croaker surimi gel

4.4.5 Folding test of croaker surimi gel with different concentration of brown seaweed (*Padina tetrastomatica*) extract

The folding tests of croaker surimi gel prepared using different concentrations of seaweed extract were presented in Table No. 4.6. In present study low folding score (2) occurred in control. The folding score (3) occurred in 0.2%, 0.4%, 0.8% of concentrations which showed no cracks occurs if folded in two but split if folded in four. The high folding score (4), showed no cracks when folded in two but a crack occurred if folded in four having 0.6% of surimi gel concentration.

Table No. 4.6 Folding test of croaker surimi gel with different concentration of brown seaweed (*Padina tetrastomatica*) extract

Sample	Brown seaweed concentrations (%)	Folding test score
A	0.0	2
B	0.2	3
C	0.4	3
D	0.6	4
E	0.8	3

5. No cracks even if folded in four

4. No Cracks occur if folded in two but a crack occurs if folded in four

3. No crack occurs if folded in two but split if folded in four

2. Cracks if folded in two

1. Splits into two if folded in two

4.4.6 Effect of dry brown seaweed (*Padina tetrastomatica*) extracts on protein solubility of croaker surimi gel

The protein solubility obtained from different concentrations of seaweed presented in Table No. 4.7. The increase in concentrations of seaweed 0.0% 0.2%, 0.4%, 0.6%, 0.8% the protein solubility decreased continuously 36.18%, 34.82, 30.82% and 40.99 respectively compared with control gel. For 0.8% seaweed concentration, protein solubility increased, but the protein solubility was lower as compared to control. The lowest protein solubility of surimi gel found in 0.6% of seaweed concentration was 30.82 % compared to control surimi gel 51.28 % (Fig. 4.7). ANOVA indicates that, significant difference was observed between the concentrations of control and 0.2%, 0.4%, 0.6 % and between control and 0.8%

($P < 0.05$).

Table No. 4.7 Effect of dry brown seaweed (*Padina tetrastomatica*) extracts on Protein solubility of croaker surimi gel

Sample	Brown seaweed concentrations (%)	Protein solubility (%)
A	0.0	51.28 ± 1.93
B	0.2	36.18 ± 3.91
C	0.4	34.82 ± 3.84
D	0.6	30.82 ± 3.63
E	0.8	40.99 ± 7.65

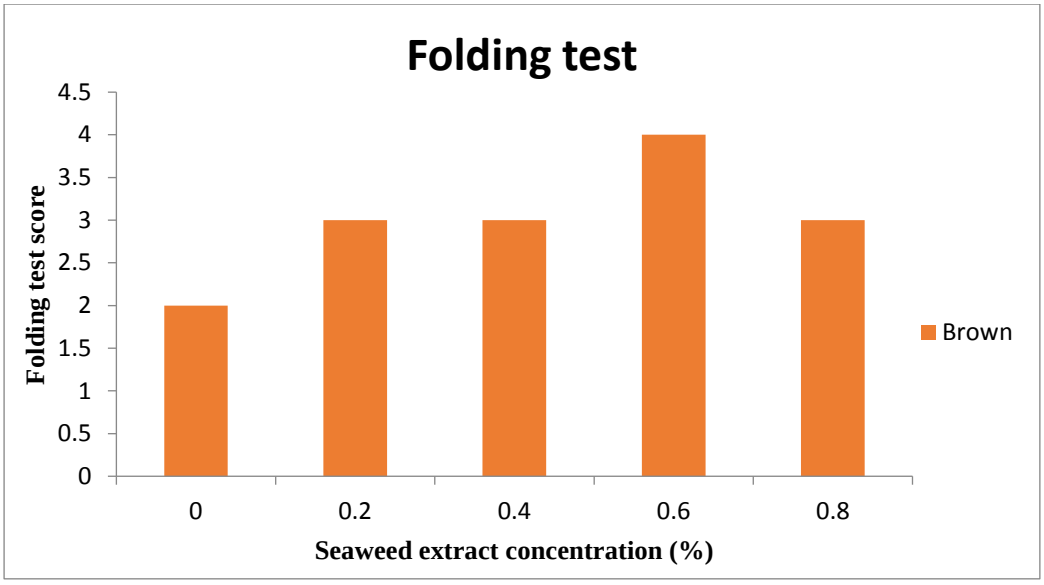

Fig. No. 4.6 Folding test of croaker surimi gel with different concentrations of brown seaweed (*Padina tetrastomatica*)extract

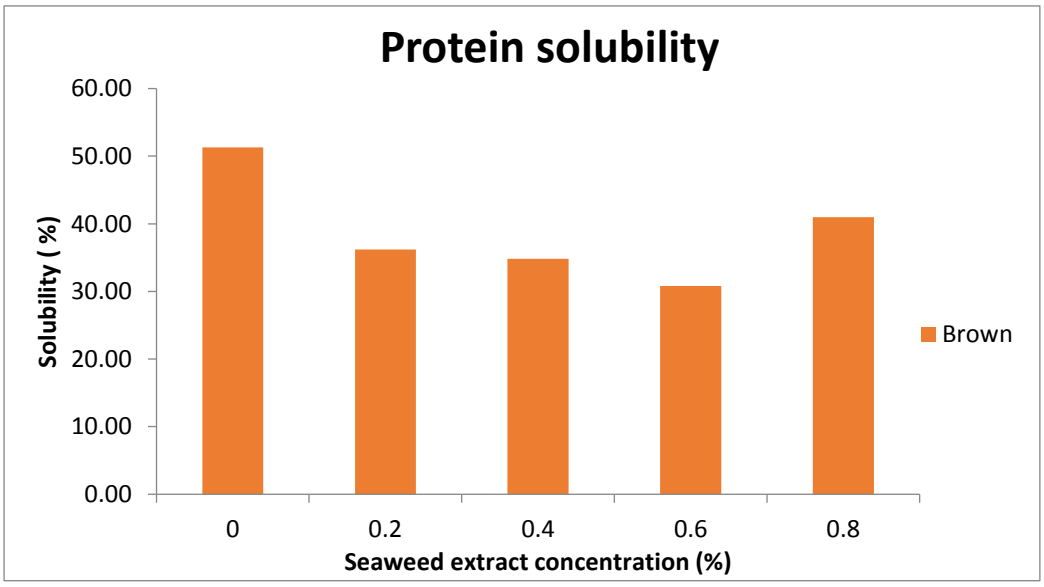

Fig. No. 4.7 Effect of dry brown seaweed (*Padina tetrastomatica*) extracts on protein solubility of croaker surimi gel

4.4.7. Effect of different concentrations of brown seaweed (*Padina tetrastomatica*) extract on the protein pattern of croaker surimi gel

The segregated protein bands with respect to molecular weight for croaker surimi gels with and without addition of different concentrations of seaweed are showed in Fig. 4.8. It was observe that, no marked changes have been found in actin band intensity in between the control surimi gel and with addition of seaweed extract.

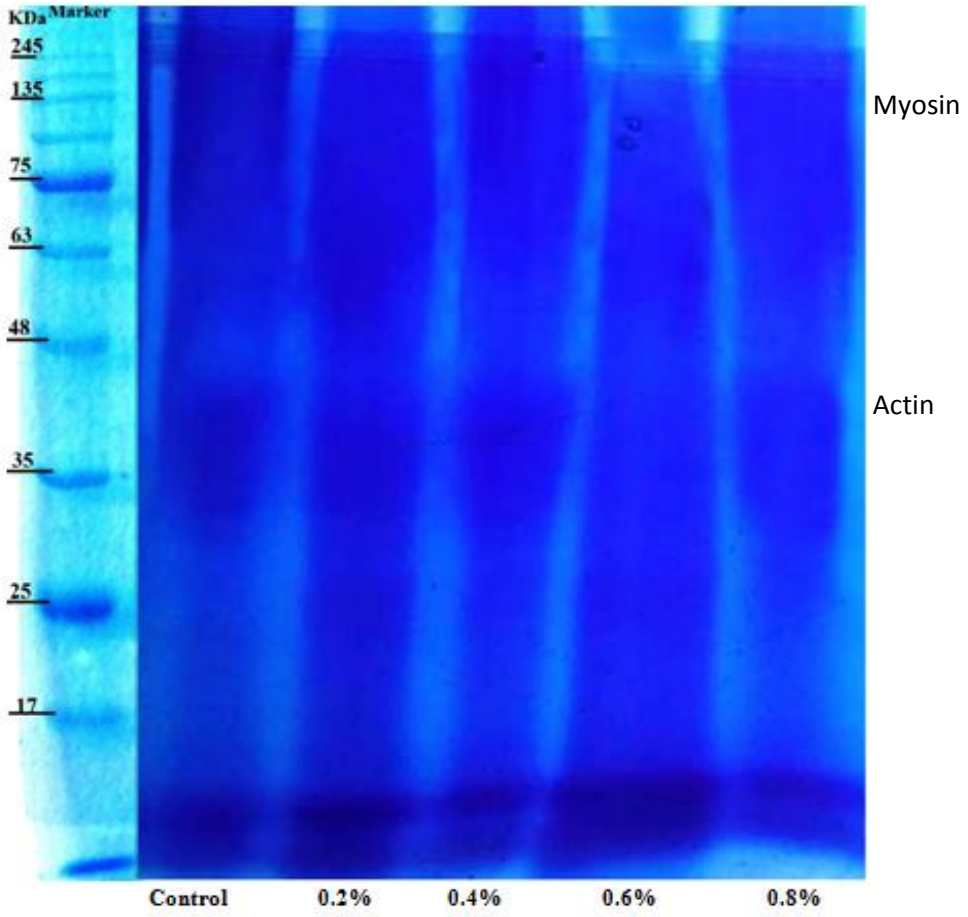

Fig.No. 4.8 Effect of different concentrations of brown seaweed (*Padina tetrastomatica*) extract on the protein pattern of croaker surimi gel

Table No 4.8 Effect of dry brown seaweed (*Padina tetrastomatica*) extract on overall croaker surimi quality

Conc. (%)	Gel strength (g/cm)	Whiteness (o)	Expressible moisture (%)	Protein solubility (%)	pH
0.0	57 ± 1.00	74.63 ± 0.79	4.47±0.02	51.28 ± 1.93	6.45 ± 0.01
0.2	61 ± 2.08	72.86 ± 0.82	2.95 ± 4.47	36.18 ± 3.91	6.45 ± 0.02
0.4	68 ± 3.06	71.94 ± 1.65	2.52 ± 0.93	34.82 ± 3.84	6.46 ± 0.02
0.6	73 ± 9.87	70.83 ± 0.67	1.63 ± 1.34	30.82 ± 3.63	6.47 ± 0.02
0.8	72 ± 7.55	69.77 ± 0.66	2.35 ± 1.07	40.99 ± 7.65	6.47 ± 0.03

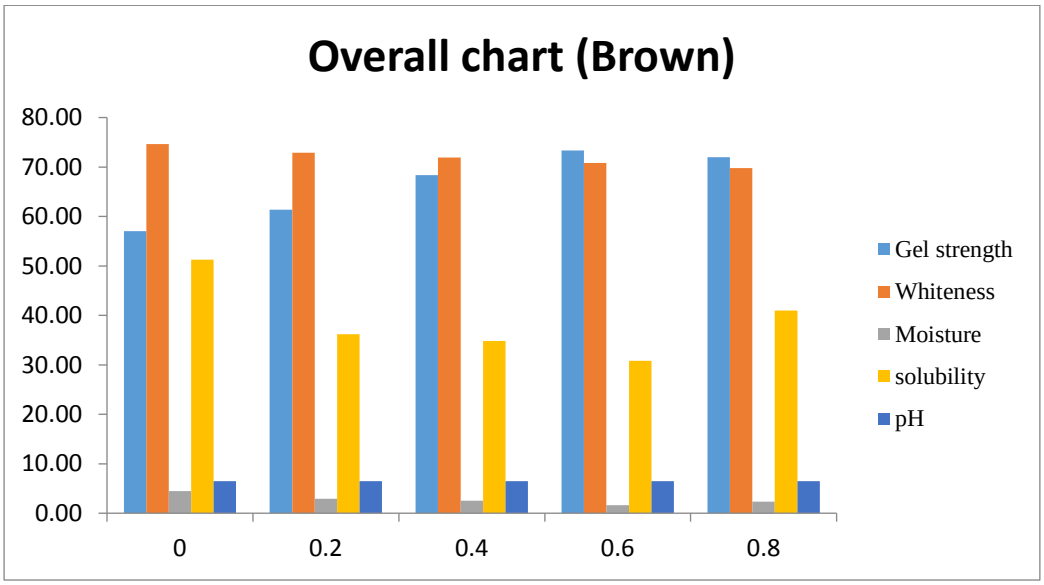

Fig. No 4.9 Effect of dry brown seaweed (*Padina tetrastomatica*) extract on overall croaker surimi quality

4.5 Green seaweed

4.5.1 Standardization of extraction of phenolic compounds from dried green seaweed (*Caulerpa peltata*)

The total phenolic content extracted using different concentrations of green seaweed (*Caulerpa peltata*) obtained with concentrations 0.5, 1.0 and 1.5 were 11.65, 15.34 and 18.14 respectively. In present study the concentration 1.0 was used for further experiment because the colour of surimi becomes dark above mentioned (1.0) concentration.

Table No. 4.9 Standardization of extraction of phenolic compounds from dried green seaweed (*Caulerpa peltata*)

Sample	Green seaweed powder concentration (g)	mg tannin/g
A	0.5	11.65 ± 0.06
B	1.0	15.34 ± 0.11
C	1.5	18.14 ± 0.06

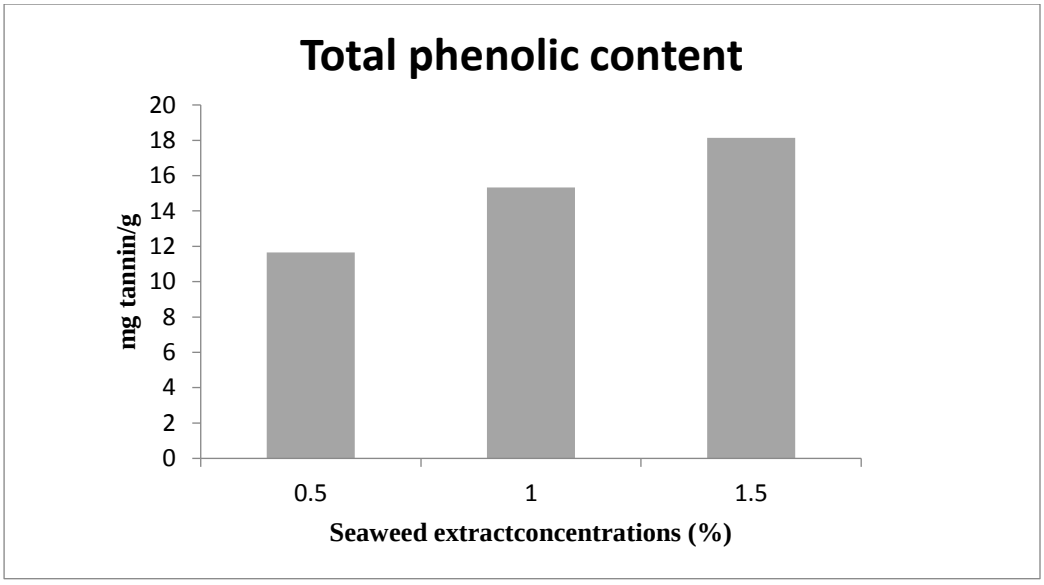

Fig.No.4.10 Standardization of extraction of phenolic compounds from dried green seaweed (*Caulerpa peltata*)

4.5.2 Effect of dry green seaweed (*Caulerpa peltata*) extract on gel strength of surimi gel

The croaker surimi gels prepared with incorporation of different concentrations of seaweed extract are 0.0% (control), 0.2%, 0.4%, 0.6% and 0.8%. All concentrations of green seaweed (*Caulerpa peltata*) extract containing total phenolic compounds of 16.75 mg tannin/g of dry seaweed powder. As, 0.0% surimi gel gives 52 g/cm, 0.2% of surimi gel gives 58 g/cm, 0.4% surimi gel gives 64 g/cm, 0.6% surimi gel gives 71 g/cm and 0.8% of surimi gel gives 67 g/cm of gel strength respectively.

From the above observation it revealed that 0.6% seaweed extract added surimi gel gives better gel strength (71 g/cm) than the other concentrations including control surimi gel. Significant difference was observed between the concentrations of control and 0.8%, 0.6 %.

Table No. 4.10 Effect of dry green seaweed (*Caulerpa peltata*) extract on gel strength of surimi gel

Sample	Green seaweed powder concentration (g)	Gel strength (g/cm)
A	0.0	52 ± 6.08
B	0.2	58 ± 3.51
C	0.4	64 ± 3.46
D	0.6	71 ± 5.51
E	0.8	67 ± 8.14

4.5.3 Effect of dry green seaweed (*Caulerpa peltata*) extracts on whiteness of surimi gel

The croaker surimi gels prepared with incorporation of different concentrations of seaweed extract are 0.0% (control), 0.2%, 0.4%, 0.6% and 0.8%. As, 0.0% surimi gel gives whiteness of 78.33°, 0.2% of surimi gel gives 76.29°, 0.4% surimi gel gives 75.67°, 0.6% surimi gel gives 74.95° and 0.8% of surimi gel gives 73.83° of whiteness respectively. The whiteness was decrease with increasing concentrations of seaweed. There was minor difference among treatments effect on whiteness of surimi gels ($P < 0.05$). Significant difference was observed between the concentrations of control and 0.2%, 0.4%, 0.6% and 0.8%.

Table No. 4.11 Effect of dry green seaweed (*Caulerpa peltata*) extract on whiteness of surimi gel

Sample	Green seaweed extracts concentrations (%)	Whiteness (°)
A	0.0	78.33 ± 0.60
B	0.2	76.29 ± 0.69
C	0.4	75.67 ± 0.53
D	0.6	74.95 ± 0.92
E	0.8	73.83 ± 0.66

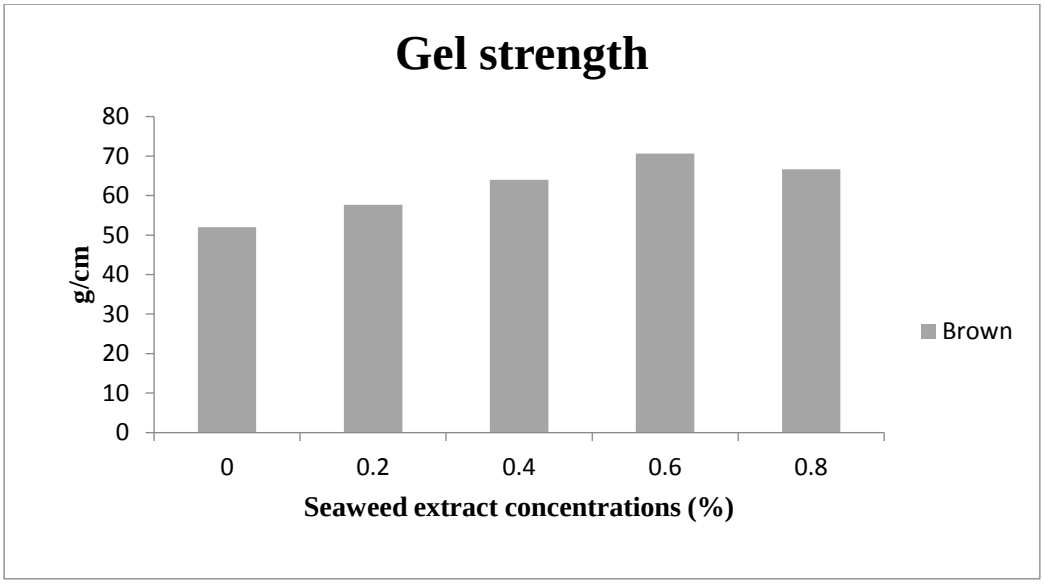

Fig. No. 4.11 Effect of dry green seaweed (*Caulerpa peltata*) extract on gel strength of surimi gel

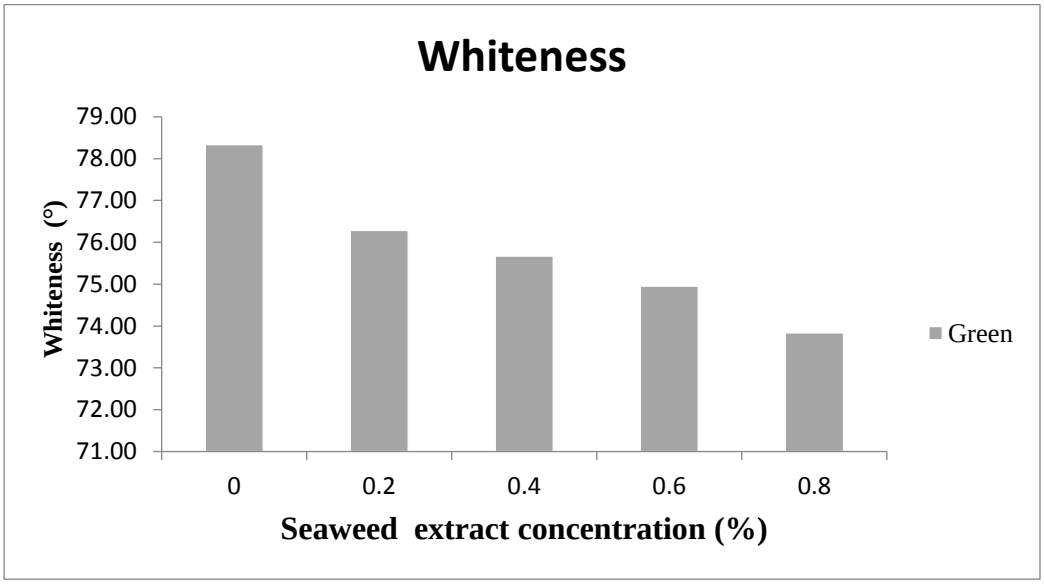

Fig. No.4.12 Effect of dry green seaweed (*Caulerpa peltata*) extract on whiteness of surimi gel

4.5.4 Effect of dry green seaweed (*Caulerpa peltata*) extracts on expressible moisture content

The croaker surimi gels prepared with incorporation of different concentrations of seaweed extract are 0.0% (control), 0.2%, 0.4%, 0.6% and 0.8%. As, 0.0% surimi gel gives expressible moisture 4.62%, 0.2% of surimi gel gives 3.13%, 0.4% surimi gel gives 2.45%, 0.6% surimi gel gives 1.28% and 0.8% of surimi gel gives 2.08% of expressible moisture respectively. ANOVA indicates that, significant difference was observed between the concentrations of control and 0.2%, 0.4%, 0.6% and 0.8% ($P < 0.05$).

Table No. 4.12 Effect of dry green seaweed (*Caulerpa peltata*) extracts on expressible moisture content

Sample	Brown seaweed concentrations (%)	Expressible moisture content (%)
A	0.0	4.62 ± 0.20
B	0.2	3.13 ± 0.81
C	0.4	2.45 ± 0.83
D	0.6	1.28 ± 0.86
E	0.8	2.08 ± 1.02

4.5.5 Effect of dry green seaweed (*Caulerpa peltata*) extracts on pH of croaker surimi gel

The croaker surimi gels prepared with incorporation of different concentrations of seaweed extract are 0.0% (control), 0.2%, 0.4%, 0.6% and 0.8% containing total phenolic compounds of 16.75 mg tannin/g of dry seaweed powder. As, 0.0% surimi gel gives pH 6.41, 0.2% of surimi gel gives 6.43, 0.4% surimi gel gives 6.43%, 0.6% surimi gel gives 6.44% and 0.8% of surimi gel gives 6.46% of pH respectively. The addition of green seaweed (*Caulerpa peltata*) extract containing phenolic compounds show the minor increase in pH with increasing concentrations, which was not significant difference compared with control surimi gel sample (P>0.05).

Table No. 4.13 Effect of dry green seaweed (*Caulerpa peltata*) extracts on pH of croaker surimi gel

Sample	Green seaweed concentrations (%)	pH
A	0.0	6.41±0.02
B	0.2	6.43±0.03
C	0.4	6.43±0.03
D	0.6	6.44±0.04
E	0.8	6.46±0.02

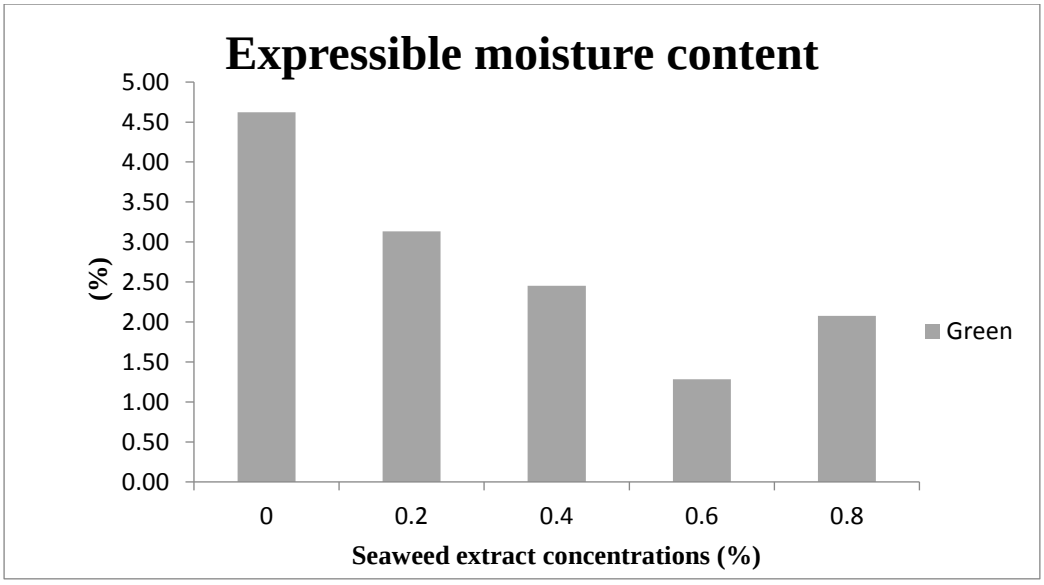

Fig. No.4.13 Effect of dry green seaweed (*Caulerpa peltata*) extracts on expressible moisture content

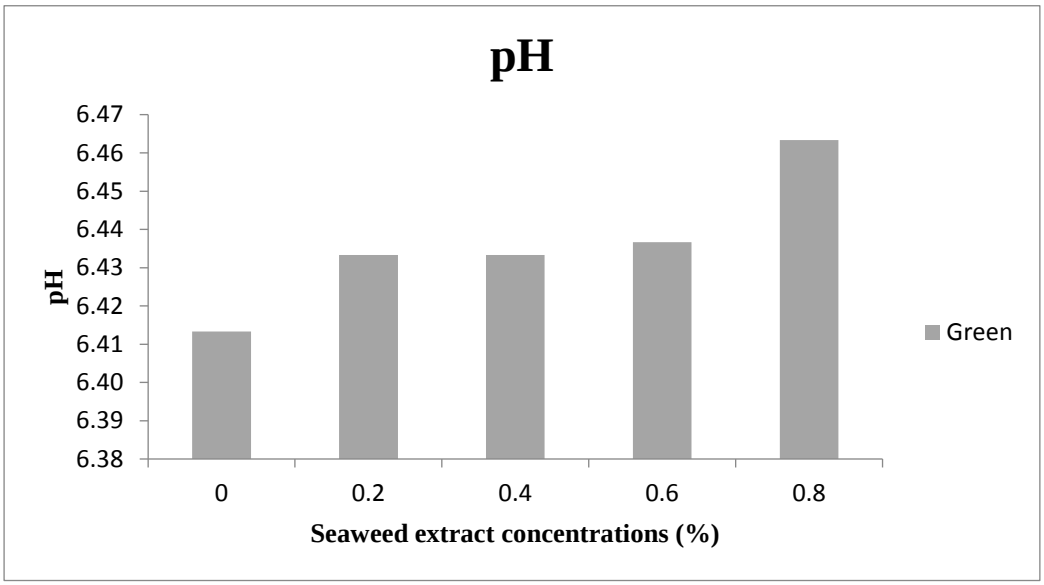

Fig. No. 4.14 Effect of dry green seaweed (*Caulerpa peltata*) extracts on pH of croaker surimi gel (P > 0.05)

4.5.6 Folding test of croaker surimi gel with different concentration of green seaweed (*Caulerpa peltata*) extract

The folding tests of croaker surimi gel prepared using different concentrations of seaweed extract were presented in Table No. 4.14. In present study low folding scores (2) occurred in control. The folding score (3) occurred in 0.2%, 0.4%, 0.8% of concentrations which showed no cracks occurs if folded in two but split if folded in four. The high folding score (4), showed no cracks when folded in two but a crack occurred if folded in four having 0.6% of surimi gel concentration.

Table No. 4.14 Folding test of croaker surimi gel with different concentration of green seaweed (*Caulerpa peltata*) extract

Sample	Brown seaweed concentrations (%)	Folding test score
A	0.0	2
B	0.2	3
C	0.4	3
D	0.6	4
E	0.8	3

5. No cracks even if folded in four.

4. No Cracks occur if folded in two but a crack occurs if folded in four.

3. No crack occurs if folded in two but split if folded in four

2. Cracks if folded in two

1. Splits into two if folded in two

4.5.7 Effect of dry green seaweed (*Caulerpa peltata*) extracts on protein solubility of croaker surimi gel

The proteinsolubility obtained from different concentrations of seaweed presented in Table No. 4.15. The increase in concentrations of seaweed 0.0% 0.2%, 0.4%, 0.6%, 0.8% the protein solubility decreased continuously 43.78%, 39.18%, 36.23% and 32.45% respectively compared with control gel. For 0.8 % seaweed concentration, protein solubility increased, but the protein solubility was lower as compared to control. The lowest protein solubility of surimi gel found in 0.6% of seaweed concentration was 36.23% compared to control surimi gel 43.73% (Fig. 4.16). ANOVA indicates that, significant difference was observed between the concentration of control and 0.2% ($P < 0.05$).

Table No. 4.15 Effect of dry green seaweed (*Caulerpa peltata*) extracts on protein solubility of croaker surimi gel

Sample	Brown seaweed concentrations (%)	Protein solubility (%)
A	0.0	43.78 ± 5.65
B	0.2	39.18 ± 3.70
C	0.4	36.23 ± 2.78
D	0.6	30.60 ± 2.44
E	0.8	32.45 ± 1.07

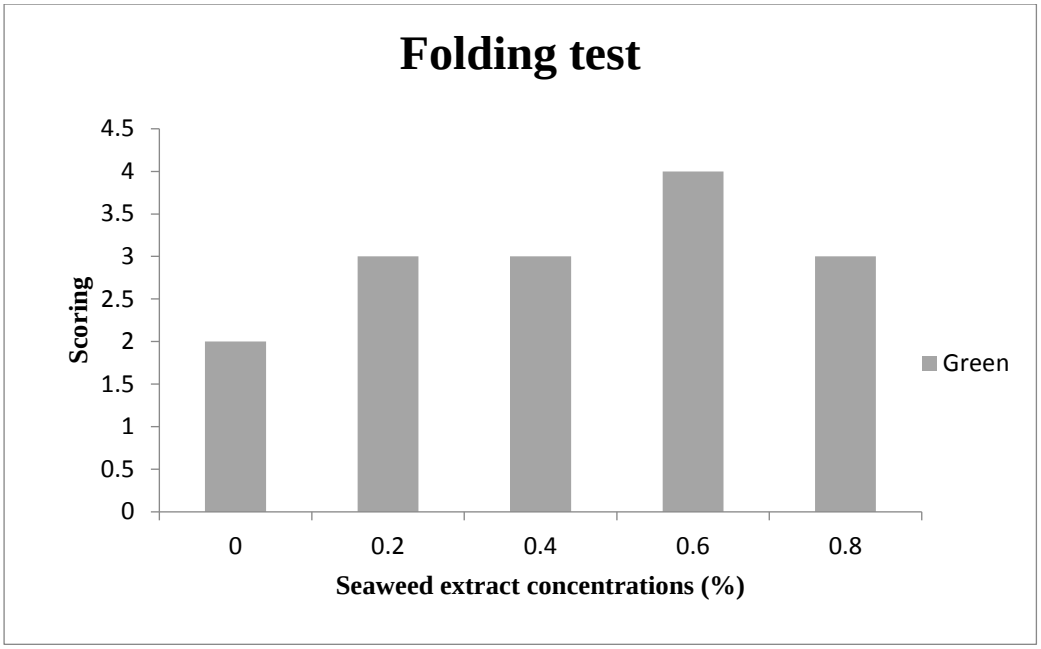

Fig. No. 4.15 Folding test of croaker surimi gel with different concentration of green seaweed (*Caulerpa peltata*) extract

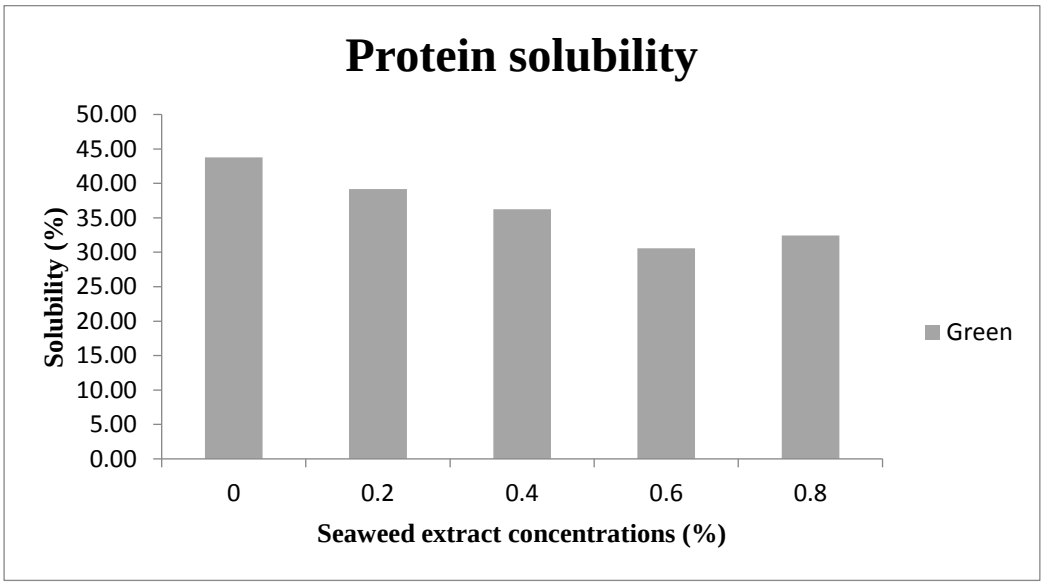

Fig. No. 4.16 Effect of dry green seaweed (*Caulerpa peltata*) extracts on protein solubility of croaker surimi gel

4.5.8 Effect of different concentrations of green seaweed (*Caulerpa peltata*) extract on the protein pattern of croaker surimi gel

The segregated protein bands with respect to molecular weight for croaker surimi gels with and without addition of different concentrations of seaweed are showed in Fig. 4.17. It was observed that, no marked changes have been found in actin band intensity in between the control surimi gel and with addition of seaweed extract.

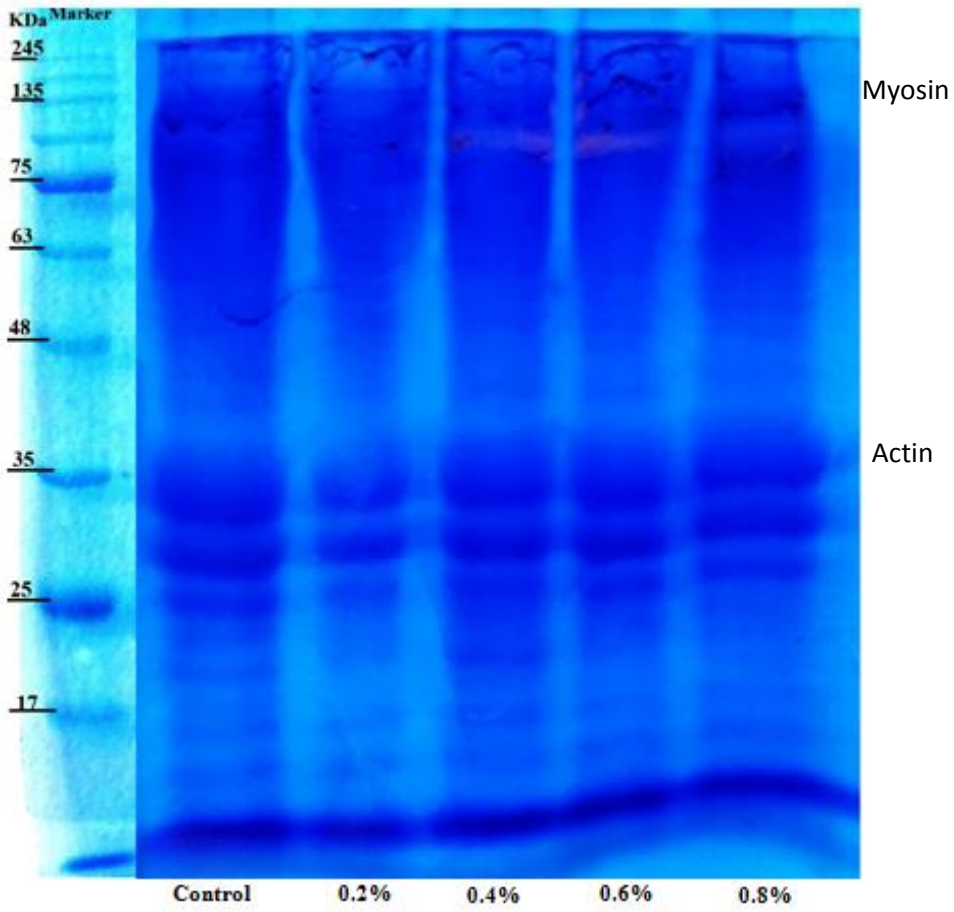

Fig.No.4.17 Effect of different concentrations of green seaweed (*Caulerpa peltata*) extract on the protein pattern of croaker surimi gel

Table No 4.16 Effect of dry green seaweed (*Caulerpa peltata*) powder extract on overall croaker surimi quality

Conc. (%)	Gel strength (g/cm)	Whiteness (º)	Expressible moisture (%)	Protein solubility (%)	pH
0.0	52 ± 6.08	78.33 ± 0.60	4.62 ± 0.20	43.78 ± 5.65	6.41±0.02
0.2	58 ± 3.51	76.29 ± 0.69	3.13 ± 0.81	39.18 ± 3.70	6.43±0.03
0.4	64 ± 3.46	75.67 ± 0.53	2.45 ± 0.83	36.23 ± 2.78	6.43±0.03
0.6	71 ± 5.51	74.95 ± 0.92	1.28 ± 0.86	30.60 ± 2.44	6.44±0.04
0.8	67 ± 8.14	73.83 ± 0.66	2.08 ± 1.02	32.45 ± 1.07	6.46±0.02

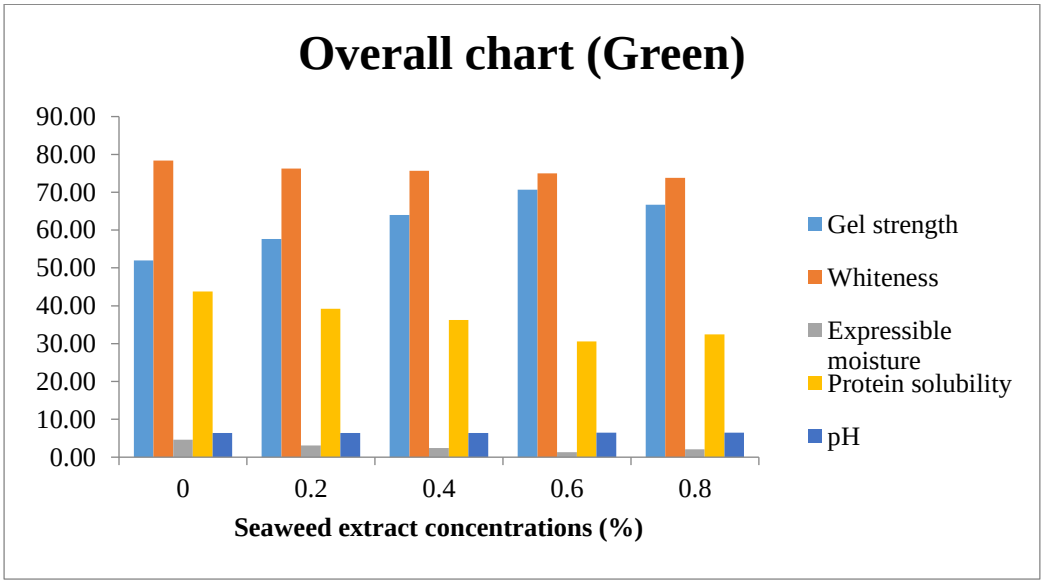

Fig. No 4.18 Effect of dry green seaweed (*Caulerpa peltata*) powder extract on overall croaker surimi quality

5.0 DISCUSSION

Surimi is primarily a stabilized fish protein used for the manufacture of surimi based seafood. It is Japanese term. Surimi is refined fish myofibrillar proteins produced through various step-by-step processes including heading, gutting, filleting, deboning, and washing, dewatering, refining, mixing with cryoprotectants, and freezing (Park, 2000). To improve the gel strength of surimi gel, various food ingredients and protein additives like egg white, beef plasma, egg albumin etc have been used in surimi industry.Phenolic compounds are present in brown and green seaweeds. Seaweeds have valuable medicinal compounds such as antibiotics, antioxidant (Lekameera et al., 2013). To improve the surimi gel property, phenolic compounds play a vital role at a proper concentration (Shitole, 2012).

5.1 Available seaweeds along Ratnagiri coast

The most available seaweeds along Ratnagiri coast were; *Sargassum tenerrimum, Chaetomorpha, Caulerpa spp., Padina spp., Gelidium spp., Dictyota dichotoma,* and *Ulva fasciata* (Redekar and Raje, 2000). The availability of seaweeds along coast decreased due to increased human population, urbanization and industrialization which lead to pollution.

5.2Quantification of total phenolic content

The total phenolic content obtained was16.75 and 15.34 mg tannin/g at 1% concentration of brown seaweed (*Padina tetrastomatica*) and green seaweed (*Caulerpa peltata*) extracts respectively. It was concluded that, the phenolic content increases with increasing quantity of brown and green seaweed extract. The phenolic compound was observed higher in brown seaweed than that of green seaweed. It may relate to species and variability in environmental factor.

Zahra et al. (2007) reported that, the total phenolic compound in seaweed (*Sargassum boveanum)* water extract was found to be 17±0.492 mg catchin equivalent (CE)/ g of dry sample. Chandini et al. (2008) found that, total phenolic compounds in *S. marginatum*, *T. conoides* and *Padina tetrastomatica* were 24.61, 49.16 and 20.04 mg GAE/g of seaweed extract (or 0.29 ,0.86 and 0.60 mg GAE/g of seaweed on dry weight basis). Hwang et al. (2010) observed that, *Sargassum hemiphyllum* with freeze dried hot water extract contained very less phenolic content, upto 0.240 mg /ml than that of sundried seaweed powder. Kuda et al.(2005) studied that the antioxidant properties of the dried brown algae (*Sargassum lomentaria*).The total phenolic contents in WE, EE and LE were 5.57, 0.42 and 4.73, respectively as mg CatE/g dry sample (DS). Shitole et al. (2014) has reported the total phenolic content extracted with water for three different sizes of brown seaweed (*Sargassum tenerrimum*) powder. It was observed that the extract prepared with 0.5g, 1.0g, 1.5g dry seaweed contained 12.70, 16.24 and 21.85 mg tannin/g of dry seaweed powder, respectively. Shitole (2012) has reported in fresh seaweed extract contains chlorophyll and other components along with phenols and in dry seaweed extract, the most of phenolic content were aggregated.

5.3Effect of dry seaweed extract (brown and green seaweed) on the quality of croaker surimi gel

5.3.1 Effect of gel strength of brown (*Padina tetrastomatica*) and green (*Caulerpa peltata*) seaweed.

Hardness of the surimi gel products is generally analyzed using compression and puncture tests. Compression force is also known as gel strength. The brown seaweed (*Padina tetrastomatica*) extract containing total phenolic compounds of 16.75 mg tannin/g of dry seaweed powder. The results revealed that 0.6% brown seaweed (*Padina tetrastomatica*) extract added surimi gel gives better gel strength (73 g/cm)

than the other concentrations including control surimi gel and 0.6% green seaweed (*Caulerpa peltata*) extract added surimi gel gives significant increase gel strength (71 g/cm) as compared to control. With 0.8% concentration of surimi gel, decrease in gel strength was observed in relation to the increasing concentrations of brown and green seaweed extracts. But the gel strength of both the seaweed extract added surimi gel was higher than that of control gel.

Balange and Benjakul (2009c) reported that, increasing quantity of ethanolic kiam wood extract (EKWE), water kiam wood extract (WKWE) and commercial tannin (CT) containing phenolic compounds, increases breaking force and deformation of gels upto a particular level. Nevertheless, the continuous decrease in breaking force and deformation were noticeable when both extracts and CT at the greater levels were added. The decreased breaking force and deformation with increasing concentrations of the extracts or commercial tannin (CT) in the study might be related with self-aggregation of phenolic compounds, leading to the loss in capability of protein cross-linking. Shitole and Balange (2014) studied that, surimi gel with 0.02% WSE showed 30% increase in gel strength, than control. Generally multidenate mechanism requires a much lower phenolic compound / protein molar ratio and thus a lower concentration of phenolic compound is needed (Haslam, 1989). In the present investigation, 0.02% concentration of WSE improved the gel strength and this might be attributed to the multidenate mechanism. However, with further increase in the concentration of WSE above 0.02%, decrease in gel strength was found. This decreasemay be related with self-aggregation of phenolic compounds, leading to the loss in capability of protein cross-linking. Shah (2018) reported that the gel strength values of surimi added with EWP at concentration of 0.5%, 1%, and 1.5% were 982 g/cm, 1281g/cm and 1560 g/cm respectively and 72, 89, and 74 g/cm at SA levels at concentrations of 0.1, 0.2 and 0.3%

respectively. Whereas higher gel strength value was observed in combination of 0.5% EWP+0.2% SA followed by combination 0.5 EWP+0.1%SA.

5.3.2Effect of dry brown seaweed (*Padina tetrastomatica*) and green seaweed (*Caulerpa peltata*) powder extract on whiteness of surimi gel

Surimi gel prepared by adding brown seaweed extract at 0.8% concentration gives whiteness 69.77° compare with the control (0.0%) 74.63°. In the same way surimi gel prepared by adding green seaweed extract at 0.8% concentration gives whiteness 73.83° compared with control (0.0%) 78.33°. The whiteness was observed to be higher in both the control samples. The whiteness of surimi gels decreases with increasing seaweed concentrations in both seaweed species. It may be associated with phenolic compounds.

Pansera et al. (2004) studied that, the process of hydrosolubilization used to extract tannins. The process operated with temperature at 100°C, the extraction process motives a hydro cracking of sugar and others organic compounds with a darkening of the final product. Benjakul et al. (2001) studied that, BPP showed detrimental effect on whiteness of surimi gel, even though it increased the gel strength with addition of porcine plasma protein decreased the whiteness of surimi gel from bigeye snapper. Balange (2009c) observed that the levels of the extracts or commercial tannins (CT) increased as the decreases in whiteness of mackerel surimi gels, it concluded that surimi gel added with EKWE at a level of 0.15% had a slight decrease in whiteness. O'Connell & Fox (2001) reported that,for discolouration in the cheese products, phenolic compounds were responsible.

5.2.3 Effect of dry brown (*Padina tetrastomatica*) and green (*Caulerpa peltata*) seaweed extracts on expressible moisture content

The zero concentrations of brown and green seaweed extract surimi gel showed expressible moisture of 4.47% and 4.62% respectively. The lowest expressible moisture content was found in 0.6% of dry brown (*Padina tetrastomatica*) and green (*Caulerpa peltata*) seaweeds extract i.e. 1.63% and 1.28% respectively. It indicates improved water-holding capacity and gel strength of surimi gel with addition of phenolic compounds

Benjakul and Visessanguan (2003) has reported that, during setting time the solubility decreased due to non-disulfide covalent bond was presumed to be a major contributor to strengthening of the gel matrix. Shitole and Balange (2014) has reported decrease in expressible moisture contents of surimi gel added with optimum WSE concentration was found in accordance with increased gel strength and indicate greater water holding capacity. Balange (2009b) observed that, the lowest expressible moisture content in gels with addition of 0.50% OTA. The results showed that, water-holding capacity of surimi gels could be improved with the addition of phenolic compounds at optimal levels. Balange (2009c) observed that, lowest expressible moisture content was found at a level of 0.15% in surimi gels added with WKWE, EKWE or CT at the optimal level. Gel network with capability of imbibing water could be obtained. The increases in expressible moisture content were found in surimi gels added with WKWE, EKWE or CT above the optimal level.

5.2.4 Effect of dry brown (*Padina tetrastomatica*) and green (*Caulerpa peltata*) seaweed extracts on pH of croaker surimi gel

In present study, the brown seaweed extracts control i.e. 0.0% concentrated surimi gel gives pH of 6.45 and 0.8% of surimi gel gives 6.47 of pH. Also, the croaker surimi gel prepared with the incorporation of different concentrations of green seaweed (*Caulerpa peltata*) extracts control i.e. 0.0% concentrated surimi gel gives pH 6.41 and

0.8% of surimi gel gives 6.46 of pH. The addition of brown seaweed (*Padina tetrastomatica*) and green seaweed (*Caulerpa peltata*) extract showed insignificant, minor increase in pH with increasing concentrations of seaweed extracts.

Shitole and Balange (2014) reported that, addition of water seaweed extract showed insignificant increase in pH with addition of different concentrations of seaweed extract. Seaweed extracts contains higher amount of phenolic compounds viz., polyphenol, having one or more aromatic rings bearing hydroxyl substituent which revealed that increase in pH (Parr and Bolwell, 2000).

5.2.5. Folding test of croaker surimi gel with different concentration of brown seaweed (*Padina tetrastomatica*) and green seaweed (*Caulerpa peltata*) extract

Thefolding test is a simple and fast method to measure the binding property of the surimi gel. In present study low folding score (2) occurred in control. The folding score (3) occurred in 0.2%, 0.4%, 0.8% of concentrations which showed no cracks even if folded in two but split if folded in four. The high folding score (4), showed no cracks when folded in two but a crack occurred if folded in four having 0.6% of surimi gel concentration of brown and green seaweed. The folding test score revealed that strongness of gel strength. The folding tests have a mutual relation to gel strength.

Kristinsson (2006) has reported that, all of the gels made from surimi, acid-aided isolates, and alkali-aided isolates, regardless of the absence or presence of cryoprotectants, passed a double-folding test without cracking, getting the best score of 5. Hema et al. (2016) observed that the high quality surimi does not show any fracture due to presence of cryoprotectants that improve the stability of myofibrillar protein and gel forming capacity. In folding test, the control surimi gel (RS-1) showed breakage and was graded as ‘B’ class. As they were made of corn starch. Starch is known to inhibit the gelation of fish proteins by competing for the available water.

5.2.6 Effect of dry brown (*Padina tetrastomatica*) and green (*Caulerpa peltata*) seaweed extracts on protein solubility of croaker surimi gel

The protein solubility was found to be decreased in brown seaweed extract added surimi gel, continuously from 36.18%, 34.82%, 30.82% and 40.99% and also for the green seaweed extract added surimi gel from 43.78%, 39.18%, 36.23% and 32.45% with the increase in concentrations of both types of seaweed extracts from 0.0%, 0.2%, 0.4%, 0.6% and 0.8% compared with the control gel. For 0.8 % seaweed concentration, protein solubility increased but the protein solubility was lower as compared to control in both seaweed species. The lowest protein solubility of surimi gel found in 0.6 % of seaweed extract concentration was 30.82 % in brown and 36.23 % in green compared to control surimi gel 51.28 % and 43.73% respectively. In present study it was found that solubility decrease with the improve gel strength.

Balange and Benjakul (2009a) have noted decrease in solubility due to the formation of protein aggregates during gelation process. Prigent (2005) has reported increase in solubility were containing urea and SDS, indicating the presence of hydrophobic and hydrogen bonds in surimi gels. Hydrogen bonds might involve in the interactions between hydroxyl groups of phenolic compounds and the nitrogen or oxygen of lysine, arginine, histidine, asparagine, glutamine, serine, threonine, aspartic acid, glutamic acid, tyrosine, cysteine and tryptophan as hydrogen acceptor. Shitole (2014) observed that solubility was found to be low in surimi gel prepared with 2% WSE. All different concentrations of seaweed extract have low solubility compared with control gel. The lower solubility of surimi gel correlated with higher gel strength.

5.2.7 Effect of different concentrations of brown and green seaweed extract on the protein pattern of croaker surimi gel

The segregation of protein bands was done with respect to molecular weight for croaker surimi gels by taking surimi, with and without added seaweed extracts of different concentrations. It was observed that, actin band intensity showed no significant changes between the control surimi gel, brown and green seaweed extract added surimi gel. It may relate to fish species and poor gel strength of it.

Benjakul et al. (2004a) has reported dark flesh have high autolytic activity, which is associated with the poor gel properties.

Conclusion

- The high phenolic compound was observed to be present in dry brown seaweed (*Padina tetrastomatica*) than green seaweed (*Caulerpa peltata*).
- The gel strength was found to be improved at 0.6% concentration of croaker surimi gel with dry brown (*Padina tetrastomatica*) and green (*Caulerpa peltata*) seaweed extract.
- The brown seaweed (*Padina tetrastomatica*) extract surimi gels have low whiteness than green seaweed (*Caulerpa peltata*) extract surimi gel.

In present study it was observed that, 0.6% concentration of brown and green seaweed extracts, increases the gel strength with decreasing expressible moisture content and protein solubility of surimi gel. Also, at the same concentration, high folding score was observed. Finally the study revealed that, gel strength was not found to be significantly improved after incorporation of appropriate concentrations of dry seaweeds extract. This may be associated with fish species, as croakers surimi has low gel strength, which was observed in control fish surimi sample.

6.0 Summary

In the present study, the brown seaweed (*Padina tetrastomatica)* and green seaweed (*Caulerpa peltata*) available along Ratnagiri coast of India were used. The availability of both the seaweed along October to March, enabled to use for study. The brown (*Padina tetrastomatica*) and green (*Caulerpa peltata*) seaweed contained total phenolic compounds. The total phenolic content increased with increasing amount of dry seaweed powder in brown and green seaweed extract. The croaker surimi gels were prepared with different concentrations of brown (*Padina tetrastomatica*) and green (*Caulerpa peltata*) seaweed. The results revealed that, 0.6% concentration to improve gel strength with both brown and green seaweed of croaker surimi gel was effective. The physical, chemical evaluations were done for prepared croaker surimi gel. The summary of results given below,

6.1 Standardization of extraction of phenolic compounds from dried brown seaweed (*Padina tetrastomatica*) **and green seaweed** (*Caulerpa peltata*)

The total phenolic content extracted using different concentrations of brown seaweed (*Padina tetrastomatica*) and green seaweed (*Caulerpa peltata*) obtained with concentrations 0.5g, 1.0g and 1.5g were 13.98, 16.75 and 21.83 and green seaweed (*Caulerpa peltata*) were 11.65, 15.34 and 18.14 respectively. In present study the concentration 1% was used for further experiment because the colour of surimi turning dark due to use of 1.5% seaweed concentration.

6.2 Effect of dry brown seaweed (*Padina tetrastomatica*) extract on the quality of croaker surimi gel

- The gel strength 73 g/cm of croaker surimi gel increased at 0.6% concentration of seaweed extract as compare to control surimi gel.

- The whiteness decreases from 74.63° to 69.77° with increasing concentrations of brown seaweed (0.0%, 0.2%, 0.4%, 0.6%, and 0.8%).
- The addition of brown seaweed (*Padina tetrastomatica*) extract of different concentrations showed the slight increase in pH with increasing concentrations.
- The expressible moisture content was decreases with increasing gel strength of surimi gel. The lower expressible moisture content 1.63% at 0.6% concentration of surimi gel.
- The lower protein solubility 30.82% observed in 0.6% concentration of seaweed extract. Protein solubility decreases with increase in gel strength.
- High value of folding score is obtained at high gel strength in surimi gel.

6.3 Effect of dry green seaweed (*Caulerpa peltata*) extract on the quality of croaker surimi gel

- The gel strength 71 g/cm of croaker surimi gel increased at 0.6% concentration of seaweed extract compare to control surimi gel.
- The whiteness decreases from 78.33° to 73.83° with increasing concentrations of brown seaweed (0.0%, 0.2%, 0.4%, 0.6%, and 0.8%).
- The addition of green seaweed (*Caulerpa peltata*) extract of different concentrations showed the slight increase in pH with increasing concentrations.
- The expressible moisture content decreases with increase in gel strength of surimi gel. The lower expressible moisture content 1.28% at 0.6% concentration of surimi gel.
- The lower protein solubility 30.60% observed in 0.6% concentration of seaweed extract. Protein solubility decreases with increasing gel strength.
- High value of folding score is obtained at high gel strength in surimi gel.

REFERENCES

Arunkumar, K., Sivakumar, S. and Rengasamy, R. (2010) Review of Bioactive Potential in Seaweeds (Marine Macroalgae): A Special Emphasis on Bioactivity of Seaweeds against Plant Pathogens. *Asian Journal of Plant Sciences.*,9: 227-240.

Balange, A. K. (2009)Enhancement of gel strength of surimi using oxidized phenolic compounds, Degree of Doctor of Philosophy in Food Science and TechnologyThesis. Prince of Songkla University, Thailand.

Balange, A. K. and Benjakul, S. (2009a) Effect of oxidized tannic acid on the gel properties of mackerel (*Rastrelliger kanagurta*) mince and surimi prepared by different washing processes. *Food Hydrocolloids.*, xxx: 1-9.

Balange, A. K. and Benjakul, S. (2009b) Effect of oxidized phenolic compounds onthe gel property of mackerel (*Rastrelliger kanagurta*) surimi. *LWT-Food Science and Technology.*, 42: 1059-1064.

Balange, A. K. and Benjakul, S. (2009c) Use of kiam wood extract as gel enhancerfor mackerel (*Rastrelliger kanagurta*) surimi. *International Journal of FoodScience & Technology*., 44: 1661-1669.

Balange, A. K. and Benjakul, S. (2009d) Enhancement of gel strength of bigeye snapper (*Priacanthus tayenus*) surimi using oxidized phenolic compounds. *Food Chemistry.*, 113: 61-70.

Balasundram, N., Sundram, K. and Samman, S. (2006) Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potentialuses. *Food Chemistry*., 99: 191–203.

Benjakul, S.,Visessanguan, W. and Srivilai, C. (2001) Gel properties of bigeye snapper *(Priacxvthus tayenus)* surimi as affected by setting and porcine plasma proteins. *JournaI of Food Quality.*24: 453-471

Benjakul, S. and Visessanguan,W. (2003) Transglutaminase-mediated setting in bigeye snapper Surimi. *Food Research International*. 36: 253–266.

Benjakul, S., Visessanguan, W., Chantarasuwan, C. (2004a) Cross-linking activity of sarcoplasmic fraction from bigeye snapper (*Priacanthus tayenus*) muscle. *Lebensm.- Wiss. u.-Technol*. 37: 79–85.

Benjakul, S., Visessanguan, W. and Chantarasuwan, C. (2004b) Effect of high temperature setting on gelling characteristic of surimi from some tropical fish. *International Journal of Food Science and Technology*. 39: 671–680.

Benjakul, S., Phatcharat, S., Tammatinna, A., Visessanguan, W., and Kishimura, H. (2008). Improvement of gelling properties of lizardfish mince as influenced by microbial transglutaminase and fish freshness. *Journal of food science*, *73*(6), S239-S246.

Cando, D., Herranz, B., Borderías, A. J., & Moreno, H. M. (2016) Different additives to enhance the gelation of surimi gel with reduced sodium content. *Food chemistry*, *196*, 791-799.

Central Marine Fisheries Research Institute (2018) Annual Report of estimated marine fish landing 24 p.

Chaijan, M., Benjakul, S., Visessanguan, W. and Faustman, C. (2004) Characteristics and gel properties of muscles from sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) caught in Thailand. *Food Research International.* 37:1021– 1030.

Chandini, S. K., Ganesan, P., and Bhaskar, N. (2008) In vitro antioxidant activities of three selected brown seaweeds of India. *Food chemistry*, *107*(2), 707-713.

Chawla, S. P., Venugopal, V., and Nair, P. M. (1996) Gelation of proteins from washed muscle of threadfin bream (*Nemipterus japonicus*) under mild acidic conditions. *Journal of Food Science*, *61*(2), 362-367.

Cheynier, V., Comte, G., Davies, K. M., Lattanzio, V., and Martens, S. (2013) Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiology and Biochemistry*, *72*, 1-20.

Connan, S. and Stengel, D. B. (2007) Environmental control of Brown Algal Phenol Production and Assessment of their Metal Binding Properties. 1st Environmental Change Institute Research Day, NUI Galway, 21stJune 2007.

Haslam, E. (1989) Plant Polyphenols: Vegetable Tannins Revisited; (Phillipson, J.D., Ayres, D. C. and Baxter, H., eds.) Cambridge University PressCambridge: 230.

Hertog, M. G., Feskens, E. J., Kromhout, D., Hollman, P. C. H., and Katan, M. B. (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The lancet*, *342*(8878), 1007-1011.

Hwang, P., Wu, C., Gau, S., Chien, S. and Hwang, D.(2010) Antioxidant andimmune-stimulating activities of hot water extract from seaweed *Sargassum hemmiphyllum.* *Journal of Marine Science and Technology*.,18: 41-46.

Jiménez-Escrig, A., Jiménez-Jiménez, I., Pulido, R., and Saura-Calixto, F. (2001) Antioxidant activity of fresh and processed edible seaweeds. *Journal of the Science of Food and Agriculture*, *81*(5), 530-534.

Klomklao, S., and Benjakul, S. (2015) Effect of trypsin inhibitor in adzuki bean (*Vigna angularis*) on proteolysis and gel properties of threadfin bream (*Nemipterus bleekeri*). *LWT-Food Science and Technology*, *63*(2), 906-911.

Kristinsson, H. G., Theodore, A. E., Demir, N., and Ingadottir, B. (2005) A comparative study between acidand alkali-aided processing and surimi processing for the recovery of proteins from channel catfish muscle. *Journal of food science*, *70*(4), C298-C306.

Kristinsson, H. G., and Ingadottir, B. (2006) Recovery and properties of muscle proteins extracted from tilapia (*Oreochromis niloticus*) light muscle by pH shift processing. *Journal of Food Science*, *71*(3), E132-E141.

Kristinsson, H. G., and Liang, Y. (2006) Effect of pH-shift processing and surimi processing on Atlantic croaker (*Micropogonias undulates*) muscle proteins. *Journal of Food Science*, *71*(5), C304-C312.

Kudo, G. E. O. R. G. E., Okada, M. I. N. O. R. U., and Miyauchi, D. A. V. I. D. (1973) Gel-forming capacity of washed and unwashed flesh of some Pacific coast species of fish. *Mar. Fish. Rev*, *35* (12), 10.

Kuda, T., Tsunekawa, M., Hishi, T. and Araki, Y. (2005) Antioxidant properties of dried 'kayamo-nori', a brown algae *Scytosiphonlomentaria* (*Scytosiphonales, Phaeophyceae*). *Food Chemistry*.89: 617–622.

Laemmli UK (1970) Cleavage of structural proteins during assembly of head of bacteriophage T4. Nature 227:680–685

Lekameera, R., Vijayabaskar, P., and Somasundaram, S. T. (2013) Evaluating antioxidant property of brown algae *Colpomenia sinuosa* (Derb. Et sol). *African Journal of Food Science*, *2*(11), 126-130.

Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., and Cheng, S. (2006) Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food chemistry*, *96*(2), 254-260.

Murugan, K., and Iyer, V. V. (2014) Antioxidant activity and gas chromatographic-mass spectrometric analysis of extracts of the marine algae, *Caulerpa peltata* and *Padina gymnospora. Indian journal of pharmaceutical sciences*, *76*(6), 548.

O'connell, J. E. and Fox, P. F. (2001).Significance and applications of phenoliccompounds in the production and quality of milk and dairy products. *Int.Dairy J.*, 11: 103-120.

Oki, T., Masuda, M., Furuta, S., Nishiba, Y., Terahara, N., and Suda, I. (2002) Involvement of anthocyanins and other phenolic compounds in radical-scavenging activity of purple-fleshed sweet potato cultivars. *Journal of Food Science*, *67*(5), 1752-1756.

Pansera, M. R., Iob, G. A., Atti-Santos, A. C., Rossato, M., Atti-Serafini, L., and Cassel, E. (2004) Extraction of tannin by Acacia mearnsii with supercritical fluids. *Brazilian Archives of Biology and Technology*, *47*(6), 995-998.

Park, J.W. (2000) Surimi and Surimi Seafood. Marcel Dekker, Inc. New York, USA:Marcel Dekker: 23-78.

Parr, A. J. and Bolwell, G.P. (2000) Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenol contentor profile. *J. Sci. Food Agric*., 80: 985-1012.

Pérez-Mateos, M., Lanier, T. C., and Boyd, L. C. (2006) Effects of rosemary and green tea extracts on frozen surimi gels fortified with omega-3 fatty acids. *Journal of the Science of Food and Agriculture*, *86*(4), 558-567.

Prigent, S. (2005) Interactions of phenolic compounds with globular proteins andtheir effects on food related functional properties.Ph.D. thesis, Wageningenh University, The Netherlands.

Ragan, M. and Glombitza, K. (1986) Phlorotannins, brown algal polyphenols. In:Round FE, Chapman DJ (eds) Progress in Phycological Research, Biopress, Bristol, UK., 4:129- 241.

Rao, M. U. (1970) The Economic Seaweeds of India, In: CMFRI Bulletin No. 20.

Rawdkuen, S. and Benjakul, S.(2008) Whey protein concentrate: Autolysis inhibition and effects on the gel properties of surimi prepared from tropical fish. *Food Chemistry*. 106: 1077–1084.

Rawdkuen, S., Sai-Ut, S., Khamsorn, S., Chaijan, M., and Benjakul, S. (2009) Biochemical and gelling properties of tilapia surimi and protein recovered using an acid- alkaline process. *Food Chemistry*, *112*(1), 112-119.

Redekar, P. D. and Raje, P. C. (2000) Alginic acid and agar-agar content from thecommon seaweeds of Ratnagiri coast (West coast of India). *Seaweed, Res. Utiln., 22(1&2)*: 41- 43.Sankar, T. V. (2009). Functional properties of fish proteins: A Review. *Fishery Technology*., 46(2): 87-98.

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glover, W. (1999) Phenolic compounds and their role in oxidative processes in fruits. *Food chemistry*, *66*(4), 401-436.

Robinson, H. W. and Hodgen, C.G. (1940) The biuret reaction in the determination of serum protein. I. A study of condition necessary for the production of the stable colour which bears a quantitative relationship to the protein concentration. *J. Biol Chem* 135:707–725

Sahoo, D. (2010) Common seaweeds of India. I. K. International Publishing House Pvt Ltd.
pp 50 -76.

Satam, S.B., Joshi V. R. and Sajidkhan I. Yusufzai (2002) Trends in production of surimi and surimi-based seafoods. *National Fisheries Journal of India.,* ISSN 0971-4529. Vol.22 No.8

Shah, S., Patange, S. B., Meshre, S. D., Koli, J. M., and Naik, S. D. (2018). Effects of egg white and sodium ascorbate on gelation properties of lesser sardine (*Sardinella spp*.) Surimi.*Journal of Entomology and Zoology.*,6(2): 2504-2507

Shaviklo, G. R. (2006) Quality assessment of fish protein isolates using surimi standard methods. *The United Nations University, fisheries training programme*.

Shitole, S. S. (2012) Gel Strength Enhancement of Indian Mackerel (*Rastrelliger kanagurta*) Surimi Using Seaweed Extract (MFSc thesis DBSKKV., Dapoli).

Shitole, S. S., Balange, A. K., and Gangan, S. S. (2014a) Use of Seaweed (*Sargassum tenerrimum*) extract as gel enhancer for lesser sardine (*Sardinella brachiosoma*) surimi. *International Aquatic Research*, *6*(1), 55.

Shitole, S. S., and Balange, A. K. (2014b) Enhancement of gel strength of surimi from Japanese Threadfin Bream (*Nemipterus japonicus* Bloch, 1791) using seaweed extract. *Fishery Technology*, *51*(2).

Silas E. G., Ramachandran Nair P. V. and Chennubhotla V. S. K. (1987) *Introduction* CMFRI Bulletin: 41.

Snedecor, G.W. and Cochran, W.G. (1967) In: Stastistical Methods, Sixth ed. Oxfordand IBH Co., New Delhi.

Solanki, J., Zofair, M., Paarmar, H., Dodia, A., Kotiya, A. and Gunalan, B. (2011) Effect of egg albumen (protein additive) on surimi prepared from lizardfish (*Saurida tumbil*) during frozen storage. *AACL Bioflux*., 4(3): 306-312.

Vimalabai, C., Prathiba, R. A. and Sumithra, P. (2004) Phenolic compounds in brown seaweeds from Tuticorin, southeast coast of India. *Seaweed Research and Utilization.*, 26 (1-2): 93-98.

Yoshie, Y., Wang, W., Hsieh, Y. and Suzuki, T. (2002) Compositional difference of phenolic compounds between two seaweeds, *Halimeda* spp. *Journal of Tokyo University of Fisheries*., 88: 21-24.

Zahra, R., Mehrnaz, M., Farzaneh, V. and Kohzad S. (2007) Antioxidant activity of extract from a brown alga, *Sargassum bovenum*. *African journal of Biotechnology*., 6 (24): 2740-2745.

Zubia, M., Payri, C. and Deslandes, E.(2008) Alginate, mannitol, phenolic compounds and biological activities of two range extending brown algae, *Sargassum mangarevense* and *Turbinaria ornata* (Phaeophyta : Fucales), fromTahiti (French Polynesia). *J. Appl. Phycol.*, 20: 1033-1043.

APPENDIX 1

One way ANOVA for effect of dry brown seaweed (*Padina tetrastomatica*) extract on gel strength of surimi gel

ANOVA						
Source of Variation	Sum of Square	Degree of freedom	Mean Square	F	P-value	F crit
Between Groups	591.6	4	147.9	4.37574	0.026576	3.47805
Within Groups	338	10	33.8			
Total	929.6	14				

(P<0.05) significant difference

Ranking for different Concentrations of brown seaweed (*Padina tetrastomatica*)

Average	57	61.33333	68.33333	73.33333	72
Rank	1	2	3	5	4

Student`s Newman Keuls (SNK) comparison test for gel strength of dried brown seaweed concentrations

Comparison	Difference	SE	q cal	P	q (0.05,10,p)	Conclusion
5VS1	16.33333	3.35659	4.86606	5	4.654	SD
5VS2	12	3.35659	3.57506	4	4.327	NSD
5VS3	5	3.35659	1.48961	3	3.877	NSD
5VS4	1.333333	3.35659	0.39723	2	3.151	NSD
4VS1	15	3.35659	4.46883	4	4.327	SD
4VS2	10.66667	3.35659	3.17783	3	3.877	NSD
4VS3	3.666667	3.35659	1.09238	2	3.151	NSD
3VS1	11.33333	3.35659	3.37645	3	3.877	NSD
3VS2	7	3.35659	2.08545	2	3.151	NSD
2VS1	4.333333	3.35659	1.29099	2	3.151	NSD

SD (P<0.05) = Significant difference, NSD (P>0.05) = No Significant difference

APPENDIX 2

One way ANOVA for effect of dry brown seaweed (*Padina tetrastomatica*) extract on whiteness of surimi gel

ANOVA						
Source of Variation	Sum of Square	Degree of freedom	Mean Square	F	P-value	F crit
Between Groups	41.9489	4	10.4872	10.6908	0.0012355	3.47805
Within Groups	9.80953	10	0.98095			
Total	51.7584	14				

(P<0.05) significant difference

Ranking for different Concentrations of brown seaweed (*Padina tetrastomatica*)

Average	74.6265	72.8555	71.9447	70.8267	69.77
Rank	5	4	3	2	1

Student`s Newman Keuls (SNK) comparison test for whiteness of dried brown seaweed concentrations

Comparison	Difference	SE	q cal	P	q (0.05,10,p)	Conclusion
5Vs1	4.8565	0.57183	8.49299	5	4.654	SD
5Vs2	3.7998	0.57183	6.6451	4	4.327	SD
5Vs3	2.6818	0.57183	4.68989	3	3.877	SD
5Vs4	1.7710	0.57183	3.09713	2	3.351	NSD
4Vs1	3.0855	0.57183	5.39586	4	4.327	SD
4Vs2	2.0288	0.57183	3.54798	3	3.877	NSD
4Vs3	0.9108	0.57183	1.59277	2	3.351	NSD
3Vs1	2.1747	0.57183	3.80309	3	3.877	NSD
3Vs2	1.1180	0.57183	1.95521	2	3.351	NSD
2Vs1	1.0567	0.57183	1.84788	2	3.351	NSD

SD (P<0.05) = Significant difference, NSD (P>0.05) = No Significant difference

APPENDIX 3

One way ANOVA for effect of dry brown seaweed (*Padina tetrastomatica*) extracts on expressible moisture content

ANOVA						
Source of Variation	Sum of Square	Degree of freedom	Mean Square	F	P-value	F crit
Between Groups	13.3164	4	3.3291	3.92827	0.03608	3.47805
Within Groups	8.47473	10	0.84747			
Total	21.7911	14				

(P<0.05) significant difference

Ranking for different Concentrations of brown seaweed (*Padina tetrastomatica*)

Average	4.46667	2.94667	2.51667	1.63333	2.35333
Rank	5	4	3	1	2

Student`s Newman Keuls (SNK) comparison test for expressible moisture content of dried brown seaweed concentrations

Comparison	Difference	SE	q cal	P	q (0.05,10,p)	Conclusion
5VS1	2.8333	0.5315	5.33084	5	4.654	SD
5VS2	2.1133	0.5315	3.97618	4	4.327	NSD
5VS3	1.95	0.5315	3.66887	3	3.877	NSD
5VS4	1.52	0.5315	2.85984	2	3.151	NSD
4VS1	1.3133	0.5315	2.471	4	4.327	NSD
4VS2	0.5933	0.5315	1.11634	3	3.877	NSD
4VS3	0.43	0.5315	0.80903	2	3.151	NSD
3VS1	0.8833	0.5315	1.66197	3	3.877	NSD
3VS2	0.1633	0.5315	0.30731	2	3.151	NSD
2VS1	0.72	0.5315	1.35466	2	3.151	NSD

SD (P<0.05) = Significant difference, NSD (P>0.05) = No Significant difference

APPENDIX 4

One way ANOVA for effect of dry brown seaweed (*Padina tetrastomatica*) extracts on pH of croaker surimi gel

ANOVA						
Source of Variation	Sum of Square	Degree of freedom	Mean Square	F	P-value	F crit
Between Groups	0.00147	4	0.00037	0.98214	0.45976	3.47805
Within Groups	0.00373	10	0.00037			
Total	0.0052	14				

(P>0.05) No significant difference

APPENDIX 5

One way ANOVA for effect of dry brown seaweed (*Padina tetrastomatica*) extracts on protein solubility of croaker surimi gel

ANOVA						
Source of Variation	Sum of Square	Degree of freedom	Mean Square	F	P-value	F c
Between Groups	740.761	4	185.19	8.7704	0.00263	3.47
Within Groups	211.154	10	21.1154			
Total	951.915	14				

(P<0.05) significant difference

Ranking for different Concentrations of brown seaweed (*Padina tetrastomatica*)

Average	51.2761	36.1827	34.8157	30.8164	40.9924
Rank	5	3	2	1	4

Student`s Newman Keuls (SNK) comparison test for protein solubility of dried brown seaweed concentrations

Comparison	Difference	SE	q cal	P	q (0.05,10,p)	Conclusion
5VS1	20.4597	2.6530	7.7119	5	4.6540	SD
5VS2	16.4604	2.6530	6.2044	4	4.3270	SD
5VS3	15.0934	2.6530	5.6892	3	3.8770	SD
5VS4	10.2836	2.6530	3.8762	2	3.1510	SD
4VS1	10.1760	2.6530	3.8357	4	4.3270	SD
4VS2	6.1767	2.6530	2.3282	3	3.8770	NSD
4VS3	4.8098	2.6530	1.8129	2	3.1510	NSD
3VS1	5.3663	2.6530	2.0227	3	3.8770	NSD
3VS2	1.3670	2.6530	0.5153	2	3.1510	NSD
2VS1	3.9993	2.6530	1.5075	2	3.1510	NSD

SD (P<0.05) = Significant difference, NSD (P>0.05) = No Significant difference

APPENDIX 6

One way ANOVA for effect of dry green seaweed (*Caulerpa peltata*) extract on gel strength of surimi gel

ANOVA						
Source of Variation	Sum of Square	Degree of freedom	Mean Square	F	P-value	F crit
Between Groups	658.4	4	164.6	5.20886	0.01571	3.47805
Within Groups	316	10	31.6			
Total	974.4	14				

(P<0.05) significant difference

Ranking for different concentrations of green seaweed (*Caulerpa peltata*)

Average	52	57.6667	64	70.6667	66.6667
Rank	1	2	3	5	4

Student`s Newman Keuls (SNK) comparison test for effect of gel strength of green seaweed (*Caulerpa peltata*)

Comparison	Difference	SE	q cal	P	q (0.05,10,p)	Conclusion
5VS1	18.66667	3.24551	5.75154	5	4.654	SD
5VS2	13	3.24551	4.00553	4	4.327	NSD
5VS3	6.666667	3.24551	2.05412	3	3.877	NSD
5VS4	4	3.24551	1.23247	2	3.151	NSD
4VS1	14.66667	3.24551	4.51906	4	4.327	SD
4VS2	9	3.24551	2.77306	3	3.877	NSD
4VS3	2.666667	3.24551	0.82165	2	3.151	NSD
3VS1	12	3.24551	3.69742	3	3.877	NSD
3VS2	6.333333	3.24551	1.95141	2	3.151	NSD
2VS1	5.666667	3.24551	1.746	2	3.151	NSD

SD (P<0.05) = Significant difference, NSD (P>0.05) = No Significant difference

APPENDIX 7

One way ANOVA for effect of dry green seaweed (*Caulerpa peltata*) extract on whiteness of surimi gel

ANOVA						
Source of Variation	Sum of Square	Degree of freedom	Mean Square	F	P-value	F crit
Between Groups	33.7491	4	8.43728	17.5525	0.00016	3.47805
Within Groups	4.80687	10	0.48069			
Total	38.556	14				

(P<0.05) significant difference

Ranking for different Concentrations of green seaweed(*Caulerpa peltata*)

Average	78.3321	76.2878	75.67	74.9533	73.8333
Rank	5	4	3	2	1

Student`s Newman Keuls (SNK) comparison test for whiteness of dried green seaweed concentrations

Comparison	Difference	SE	q cal	P	q (0.05,10,p)	Conclusion
5Vs1	4.4988	0.40029	11.2388	5	4.654	SD
5Vs2	3.3788	0.40029	8.44084	4	4.327	SD
5Vs3	2.6621	0.40029	6.65046	3	3.877	SD
5Vs4	2.0443	0.40029	5.10716	2	3.351	SD
4Vs1	2.4544	0.40029	6.13168	4	4.327	SD
4Vs2	1.3344	0.40029	3.33368	3	3.877	NSD
4Vs3	0.6178	0.40029	1.5433	2	3.351	NSD
3Vs1	1.8367	0.40029	4.58839	3	3.877	SD
3Vs2	0.7167	0.40029	1.79039	2	3.351	NSD
2Vs1	1.1200	0.40029	2.798	2	3.351	NSD

SD (P<0.05) = Significant difference, NSD (P>0.05) = No Significant difference

APPENDIX 8

One way ANOVA for effect of dry green seaweed (*Caulerpa peltata*) extracts on expressible moisture content

ANOVA						
Source of Variation	Sum of Square	Degree of freedom	Mean Square	F	P-value	F crit
Between Groups	19.027	4	4.75676	7.53041	0.00457	3.47805
Within Groups	6.31673	10	0.63167			
Total	25.3438	14				

(P<0.05) significant difference

Ranking for different Concentrations of green seaweed (*Caulerpa peltata*)

Average	4.62333333	3.1333333	2.45333	1.28333	2.07667
Rank	5	4	3	1	2

Student`s Newman Keuls (SNK) comparison test for effect of dry green seaweed (*Caulerpa peltata*) extract on expressible moisture content

Comparison	Difference	SE	q cal	P	q (0.05,10,p)	Conclusion
5VS1	3.34	0.4589	7.27882	5	4.654	SD
5VS2	2.55	0.4589	5.54992	4	4.327	SD
5VS3	2.17	0.4589	4.72905	3	3.877	SD
5VS4	1.49	0.4589	3.24714	2	3.151	SD
4VS1	1.85	0.4589	4.03168	4	4.327	SD
4VS2	1.06	0.4589	2.30278	3	3.877	NSD
4VS3	0.68	0.4589	1.48191	2	3.151	NSD
3VS1	1.17	0.4589	2.54977	3	3.877	NSD
3VS2	0.38	0.4589	0.82086	2	3.151	NSD
2VS1	0.79	0.4589	1.7289	2	3.151	NSD

SD (P<0.05) = Significant difference, NSD (P>0.05) = No Significant difference

APPENDIX 9

One way ANOVA for effect of dry green seaweed (*Caulerpa peltata*) extracts on pH of croaker surimi gel

ANOVA						
Source of Variation	Sum of Square	Degree of freedom	Mean square	F	P-value	F crit
Between Groups	0.00383	4	0.00096	1.61236	0.24581	3.47805
Within Groups	0.00593	10	0.00059			
Total	0.00976	14				

(P>0.05) No significant differences

APPENDIX 10

One way ANOVA for effect of dry green seaweed (*Caulerpa peltata*) extract on protein solubility of croaker surimi gel

ANOVA						
Source of Variation	Sum of Square	Degree of freedom	Mean Square	F	P-value	F crit
Between Groups	334.461	4	83.6153	6.91595	0.00616	3.47805
Within Groups	120.902	10	12.0902			
Total	455.363	14				

(P<0.05) significant difference

Ranking for different Concentrations of green seaweed (*Caulerpa peltata*)

Average	43.7783	39.1812	36.2267	30.5975	32.4473
Rank	5	4	3	1	2

Student`s Newman Keuls (SNK) comparison test for protein solubility of dried green seaweed concentrations

Comparison	Difference	SE	q cal	P	q (0.05,10,p)	Conclusion
5VS1	13.1808	2.0075	6.5658	5	4.654	SD
5VS2	11.3310	2.0075	5.6443	4	4.327	SD
5VS3	7.5517	2.0075	3.7617	3	3.877	NSD
5VS4	4.5971	2.0075	2.2900	2	3.151	NSD
4VS1	8.5837	2.0075	4.2758	4	4.327	NSD
4VS2	6.7339	2.0075	3.3544	3	3.877	NSD
4VS3	2.9546	2.0075	1.4718	2	3.151	NSD
3VS1	5.6291	2.0075	2.8040	3	3.877	NSD
3VS2	3.7793	2.0075	1.8826	2	3.151	NSD
2VS1	1.8498	2.0075	0.9214	2	3.151	NSD

SD (P<0.05) = Significant difference, NSD (P>0.05) = No Significant difference