



Effect of Indian Brown Seaweed (*Sargassum* spp) on the Quality of Kodo Millet Incorporated Chicken Nuggets

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Abstract

*The current study envisages the antioxidant fortification in Kodo millet incorporated functional chicken meat nuggets by incorporation of *Sargassum tenerrimum*, an Indian Brown Seaweed at three levels viz., 0.25% (T1), 0.5% (T2) and 0.75% (T3). Product characteristics like emulsion stability and product yield; physico-chemical, organoleptic quality characteristics and invitro antioxidant activity by DPPH radical scavenging activity were assessed. The statistical analysis of the results using SPSS software (IBM) revealed non-significant improvement of product and physico-chemical characteristics in the treatment nuggets reflected that the inclusion of the brown seaweed did not affect the quality characteristics of seaweed incorporated chicken meat nuggets. Significant decrease in L* and a* and increase in b* values were observed due to the pigment phycopine, a brown pigment and xanthophylls, a yellow pigment which contributes to the dark colour to the product. Significant increase in hardness and adhesiveness of treated nuggets were observed with increasing levels of incorporation of *S. tenerrimum*. The significant (P<0.01) increase in the antioxidant activity revealed the stability of antioxidant potential of *S. tenerrimum* in cooked nuggets. Based on the results of the organoleptic scores, the inclusion of 0.5 per cent of *S. tenerrimum* was concluded as an optimum level to be incorporated for the acceptability of the chicken nuggets to achieve the functional benefits of antioxidant activity and health of the consumers.*

Keywords: DPPH Radical Scavenging Activity, Indian Brown Seaweed, Nugget, Organoleptic Quality, Physico-Chemical Quality, *Sargassum* Species

Introduction

With the trend of consumers shift towards health conferring foods other than those meeting their nutritional needs as a part of their regular consumption, the “functional foods” gained momentum. Consumers demands have been a trigger and driving force to the meat industry to undergo transformation towards the production of meat based functional foods. Meat carries excellent potential of bioactive functional ingredients which could be delivered in varied attractive forms without changing the consumers life style eating habits. Of the various strategies for developing functional meat foods, processing strategy is a skeptical approach to modulate several endogenous and exogenous compounds to exercise health promoting physiological functions. Several non-meat ingredients from plant and animal sources have been used to reformulate meat products with healthy compounds (Arihara, 2006). An example of such healthy non meat ingredient that offers health benefits due to dietary fiber content is Kodo millet. Apart from the health benefits, it also offers functional benefits by influencing the colour, texture, sensory and nutritional quality of processed foods.

Seaweeds are the rich marine resource possessing many functional and health promoting antioxidants, antibacterial bioactive compounds but are the under exploited for processing functional food. Pharmaceutical and Chemical industries have been screening the bioactive substances from the marine organisms, especially from seaweeds (Arunkumar *et al.*, 2010). Industrial exploitation of seaweeds as a source of alginates, agar agar and carageenan has gained popularity (Rao and Mantri, 2006) and over ruled the traditional practice of its consumption as food even before its potential health benefits are on the limelight. Seaweeds, apart from their known nutritional containment of essential amino acids, unsaturated fatty acids, vitamins and minerals, the bioactive compounds carrying antioxidant property attracts its use in health promotion through novel functional food processing. Seaweeds have recently received significant attention for their potential as natural antioxidants (Vairappan *et al.*, 2001). Seaweed polysaccharides are utilised to stabilise food products against degradation, staling upon heating or cooling/freezing. These applications also act to improve the consumer acceptability of such products, as well as extending the shelf-life. The simple way of incorporation of the bioactive compounds is design of formulation by direct addition during meat processing (Khan *et al.*, 2011). Utilisation of seaweeds in functional meat products have been documented by various researchers (Chun *et al.*, 1999; Lopez *et al.*, 2009; Lopez *et al.*, 2010; Choi *et al.*, 2012; Moroney *et al.*, 2013; Choi *et al.*, 2015, Singh *et al.*, 2017). Consumers demand for “green” products will also be satisfied upon the utilisation of seaweeds in food products (Gupta and Abu Ghannam, 2011).

Sargassum, a genus of brown seaweed, belonging to Class- Phaeophyceae, Order- Fucales, Family- Sargassaceae and commonly known as gulf-weed or sea holly is one of the abundant commercial brown seaweed available in the west coast of India. Sargassum possesses many pharmacological properties, hence it has been considered as a medicinal food of the twenty-first century (Yende *et al.*, 2014) but its utilisation in food stays below the optimal. Studies utilising *S. tenerrimum* in food products are very sparsely available in the literature, hence, the current study envisages design and optimisation of inclusion of *S. tenerrimum* seaweed in the kodo millet incorporated chicken nuggets based on the quality characteristics and the anti-oxidant activity in the meat product with the target objective of providing functional benefits to consumers.

Materials and Methods

Collection and Processing of Seaweed

Sargassum tenerrimum J. Agardh were collected from the Mandapam coast, Gulf of Mannar, India during the month of April- June 2019 and were identified according to the characteristics and identification keys in the taxonomic publications by the Botanical Survey of India, Coimbatore. Collected seaweeds were transported in ice packed thermocole boxes to the laboratory. Samples were washed thoroughly several times with fresh water to remove sand, salt, epiphytes and any associated debris and shade dried at room temperature ($34 \pm 2^\circ\text{C}$) with good controlled air condition carefully for about 24 hours. The shade dried seaweeds were powdered, packed in polythene covers and stored in freezer at -20°C until used for further experiments.

Preparation of Chicken Nuggets

Emulsion was prepared by mixing the minced meat obtained from hygienically dressed and deboned chicken with other ingredients like salt, sodium tripolyphosphate, sodium nitrite, spice mix, green condiments in a commercial

food blender. Binders like kodo millet flour (KMF) was included @ 7 percent in control and treated nuggets and refined wheat flour (RWF) was included at different levels replaced with *S. tenerrimum* powder (Table 1). The selection of different inclusion levels of seaweed were based on the results of preliminary sensory analysis and the assessment of invitro antioxidant activity by DPPH radical scavenging activity. Control nuggets formulation consist of 10% refined wheat flour alone. Ice flakes were added at intervals during emulsification process to maintain the temperature of emulsion below 15°C. Refined sunflower oil was added slowly during the initial stages for stable emulsion matrix formation.

Table 1: Formulation of kodo millet incorporated functional chicken meat nuggets with *S. tenerrimum*

S. No.	Ingredients	Percentage (w/w)			
		Control	T1	T2	T3
1	Lean chicken meat	69.52	69.52	69.52	69.52
2	Salt	1.45	1.45	1.45	1.45
3	Sodium tri poly phosphate	0.22	0.22	0.22	0.22
4	Sodium nitrite	0.01	0.01	0.01	0.01
5	Oil	7.23	7.23	7.23	7.23
6	Spice mix	1.45	1.45	1.45	1.45
7	Condiment mix (Onion: Garlic-3:1)	2.89	2.89	2.89	2.89
8	Ice flakes	7.23	7.23	7.23	7.23
9	Kodo millet flour	-	7	7	7
10	Refined wheat flour	10	2.75	2.5	2.25
11	<i>S. tenerrimum</i> powder	-	0.25	0.5	0.75
		100	100	100	100

The prepared emulsion was stuffed tightly in aluminium boxes (25cmx10cmx10cm) and steam cooked at 121° C for 30 minutes. The cooked meat loaves were cooled, sliced into nuggets. The nuggets were subjected to evaluation of product characteristics like emulsion stability and product yield; physico- chemical quality characteristics like emulsion pH, product pH and water holding capacity, colour and texture profile analysis; organoleptic characteristics like appearance, flavour, texture, juiciness and overall acceptability and invitro antioxidant activity by DPPH radical scavenging activity.

Product Characteristics

Emulsion Stability (%)

The emulsion stability was determined as per the method outlined by Baliga and Madaiah (1971) and modified by Kondaiah *et al.* (1985). Packets containing fifteen grams of emulsion were submerged in a water bath at constant temperature of 80° C for 20 minutes, the released fluid was drained and the sample was weighed. The emulsion stability is calculated by using the equation-

$$Emulsion\ stability = \frac{Weight\ of\ emulsion\ after\ heating}{Weight\ of\ raw\ emulsion} \times 100$$

Product Yield (%)

Product yield was determined by the method outlined by Verma *et al.* (2012). The emulsion weight and the product weight were recorded and the product yield was calculated by using the equation-

$$Product\ yield(per\ cent) = \frac{Weight\ of\ product\ after\ cooking}{Weight\ of\ product\ before\ cooking} \times 100$$

Physico-Chemical Quality Characteristics

pH

The emulsion and product pH of the chicken nuggets sample was estimated by immersing the emulsion and product

slurry respectively in a precalibrated digital pH meter (Cyberscan pH 510, Merck). The slurry was prepared by homogenising 5g of sample with 45 ml of distilled water in a laboratory blender for one minute.

Water Holding Capacity (%)

Water holding capacity (WHC) was estimated as per the method prescribed by Wardlaw *et al.* (1973). Twenty gram of the sample was mixed with 30 mL of 0.6 M NaCl solution and homogenised for 2 min in a homogeniser. The slurry was held at 4°C for 15 min for equilibration of salt and was then stirred for 1 min on a shaker and centrifuged at 5000 rpm for 10 min at 9°C in a refrigerated centrifuge (Thermo Scientific, Sorvall ST8 R Centrifuge model). The supernatant volume was measured and difference between the added and decanted solution was expressed as percentage of the initial weight of sample.

$$WHC(\%) = \frac{\text{Volume of solution taken} - \text{Volume of decanted solution}}{\text{Weight of the sample}} \times 100$$

Hunter Colour Analysis

Colour was measured in triplicates for each sample using Hunter lab Mini scan XE plus Spectro- colorimeter having an illuminant of D65/10° (Model No. 45/O-L, Reston Virginia, USA) (Bindu *et al.*, 2007). The absorbance was expressed as L* (brightness), a*(redness) and b* (yellowness). The hue (relative position of colour between redness and yellowness) and chroma (colour intensity) was calculated as follows-

$$\text{Hue} = \tan^{-1} (b^*/a^*)$$

$$\text{Chroma} = \sqrt{(a^*)^2 + (b^*)^2}$$

Texture Profile Analysis (TPA)

Texture profile analysis was conducted in triplicate for each sample using a Stable Microsystems Texturometer (model TX_HD plus texture analyser attached to a software texture expert system) by the procedure outlined by Bourne (1978). Nugget samples of 2 cm³ in triplicate/trial were compressed twice by a cylindrical probe (P75) of 75 mm diameter to 50% of their original height to form a “two bite” work force compression curve at cross head speed of 50mm/min. The pre-test, test and post-test speed were 1mm/sec, 5mm/sec and 5mm/sec respectively. The parameters assessed are hardness (N)= maximum force required to compress the sample, springiness (cm)= ability of the sample to recover to its original shape after the deforming force was removed, cohesiveness (ratio)= extent to which the sample could be deformed prior to rupture (A2/A1, A1 being the maximum force required for the first compression and A2 being the maximum force required for the second compression), gumminess (N)= force to disintegrate a semisolid meat sample for swallowing (hardness x cohesiveness) and chewiness(Ncm)= work to masticate the sample for swallowing (springiness x gumminess).

Organoleptic Analysis

The organoleptic quality evaluation of control and treated chicken nuggets were conducted for the sensory characteristics like appearance, flavor, texture, juiciness and overall acceptability by a semi trained panel of nine members on a nine-point hedonic scale (Keeton *et al.*, 1983).

Anti-Oxidant Capacity (DPPH Radical Scavenging Activity)

The scavenging ability of *S. tenerrimum* of the 1, 1 diphenylpicrylhydrazyl (DPPH) radical was estimated following the modified method of Jung *et al.* (2010). Ten gram of the sample was mixed with 20 ml of ethanol for 2 min and blended in a homogeniser. The slurry was filtered using Whatman filter paper No 42 to obtain a clear extract. A 200 µL sample aliquot was mixed with 800 µL distilled water in a test tube and 1 mL methanolic DPPH solution (0.2 mM) was added and properly mixed using a vortex, The mixture was kept undisturbed at room temperature (20–22 °C) for 30 min. The control was prepared by mixing 1 mL of distilled water and 1 mL of methanolic DPPH solution (0.2 mM) in a test tube. Ethanol was used as blank. The absorbance of the solution was measured at 517 nm using a UV-Vis spectrophotometer (Agilent Technologies, Model. Cary 60).

$$\text{DPPH Scavenging activity (\%)} = (\text{Ab}_{\text{control}} - \text{Ab}_{\text{sample}} / \text{Ab}_{\text{control}}) \times 100$$

$\text{Ab}_{\text{control}}$ - Absorbance of the control (DPPH solution without sample)

$\text{Ab}_{\text{sample}}$ - Absorbance of the test sample (DPPH solution plus test sample)

Statistical Analysis

The experiments were conducted in six replicates (n=6). The values were analysed by the method outlined by Snedecor and Cochran (1989) using SPSS software by one-way analysis of variance (ANOVA) and the significance of the mean was accomplished by Duncans multiple range test.

Results and Discussion

Product Characteristics

Despite the statistical similarity between control and treatment nuggets for emulsion stability and product yield, numerical increase signifies the influence of incorporation of seaweed in chicken nuggets. Increase in the ionic strength and water binding capacity due to the incorporation of seaweed containing salt content and the dietary fiber content is attributed to the improvement in emulsion stability. This improvement in emulsion stability have been documented by Jimenez-Colmenero *et al.* (2010) in pork frankfurters and Choi *et al.*, 2015 in pork patties. The increase in product yield of functional chicken nuggets is observed to have positive correlation with the increase in emulsion stability (Table 2). Improvement in the product yield has been reported by Kumar and Sharma (2004) in carageenan treated low fat pork patties.

Table 2: Mean±SD of the product characteristics of *S. tenerrimum* incorporated functional chicken nuggets

Groups	Emulsion stability	Product Yield
Control	96.75±1.27	96.89±1.68
T1	97.32±0.91	96.74±1.34
T2	97.35±1.22	96.93±1.46
T3	98.49±1.25	97.07±1.59
'F' Value	0.79	0.05

Physico-Chemical Quality Characteristics

Non-significant difference between control and treatment nuggets for physico-chemical quality characteristics like Emulsion pH, Product pH and water holding capacity (Table 3) was observed. Increase of pH values in emulsion and product treatments reflects the alkaline nature of the seaweed powder being utilised.

Table 3: Mean±SD of the physico-chemical characteristics of *S. tenerrimum* incorporated functional chicken nuggets

Groups	Emulsion pH	Product pH	WHC
Control	6.28±0.07	6.40±0.03	38.27±5.11
T1	6.29±0.06	6.35±0.08	40.93±3.65
T2	6.29±0.05	6.39±0.01	42.21±4.86
T3	6.30±0.05	6.39±0.02	42.61±1.20
'F' Value	0.16	1.2	1.43

Similar increase was observed by several researchers in meat products and emulsion systems using seaweed (Cofrades *et al.*, 2008; Lopez *et al.*, 2009; Kim *et al.*, 2010; Das *et al.*, 2013 and Choi *et al.*, 2015). The values of product pH were observed to be higher than the emulsion pH of all the groups. The increase in emulsion stability recorded with the increase in the levels of incorporation of *S. tenerrimum* could be attributed to the water binding and fat binding capacity of seaweed in the product. Numerical increase in WHC among treatments was observed to be positively correlated to emulsion stability. The influence of seaweed on the physico-chemical properties especially WHC may be related to the protein associated with the cell wall and the dietary fiber contained in the

powdered seaweed (Wong and Cheung, 2000). Meat products are reported to have better water binding properties with seaweed (Cofrades *et al.*, 2011) and carageenan incorporation (Das *et al.*, 2013).

Hunters Colour Values

Significant decrease ($P < 0.01$) in lightness values (L^*) and redness (a^*) and increase in yellowness (b^*) values with increase in the inclusion levels of *S. tenerrimum* in treatment nuggets (Table 4) were recorded. This may be due to the influence of pigments like phycophine, a brown pigment and xanthophylls, a yellow pigment in addition to the chlorophyll that endows the product with a variety of darkening from yellow to brown, masking the green of the chlorophyll. Similar decrease in L^* and a^* and increase in b^* value were observed by Cofrades *et al.* (2008); Choi *et al.* (2012); Jeon and Choi, (2012) and Choi *et al.* (2015) in brown seaweed incorporated meat products and emulsion systems.

Table 4: Mean \pm SD of the colour of *S. tenerrimum* incorporated functional chicken nuggets

Groups	L^*	a^*	b^*	Hue	Chroma
Control	65.04 ^d \pm 1.18	6.68 ^c \pm 0.12	26.21 ^c \pm 0.630	0.9990 ^{ab} \pm 0.0000	27.04 ^c \pm 0.61
T1	61.98 ^c \pm 0.83	6.24 ^{bc} \pm 0.47	21.47 ^a \pm 1.15	0.9990 ^{ab} \pm 0.0006	22.15 ^a \pm 1.16
T2	59.00 ^b \pm 1.35	5.84 ^{ab} \pm 0.37	24.33 ^b \pm 1.05	0.9997 ^b \pm 0.0005	25.02 ^b \pm 1.08
T3	53.93 ^a \pm 1.32	5.46 ^a \pm 0.45	24.40 ^b \pm 0.60	0.9988 ^a \pm 0.0008	24.22 ^b \pm 0.49
'F' Value	95.16**	11.58*	29.43**	2.66*	31.65**

Means bearing different superscripts between columns differ significantly (*- $P \leq 0.05$; **- $P \leq 0.01$)

Texture Profile Analysis

Significant ($P < 0.01$) increase in hardness, adhesiveness, gumminess and chewiness (Table 5) was observed. Similar observations except for adhesiveness were recorded by Cofrades *et al.* (2008) in meat emulsion systems and Das *et al.* (2013) in chicken patties. Though, not different statistically, numerical decrease in springiness and cohesiveness were observed with increasing levels of inclusion of *S. tenerrimum* among treated nuggets. Decrease in cohesiveness was also reported by Das *et al.* (2013). Contrary findings of decreased hardness, gumminess and chewiness were reported by Choi *et al.* (2012 and 2015) in seaweed incorporated pork patties and frankfurters respectively. This variation in textural attributes can be explained by the differences in proportion, composition and characteristics of dietary fiber contained in the seaweed.

Table 5: Mean \pm SD of the texture profile of *S. tenerrimum* incorporated functional chicken nuggets

Groups	Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess	Chewiness
Control	21.17 ^b \pm 1.03	0.17 ^b \pm 0.03	0.45 \pm 0.03	0.86 \pm 0.00	18.35 ^b \pm 0.90	8.11 ^a \pm 0.75
T1	17.92 ^a \pm 0.87	0.10 ^a \pm 0.04	0.54 \pm 0.07	0.87 \pm 0.04	15.29 ^a \pm 0.50	6.86 ^a \pm 1.80
T2	20.99 ^b \pm 1.25	0.11 ^a \pm 0.02	0.46 \pm 0.13	0.85 \pm 0.04	17.89 ^b \pm 0.50	8.14 ^a \pm 2.41
T3	26.31 ^c \pm 3.40	0.11 ^a \pm 0.02	0.45 \pm 0.12	0.84 \pm 0.02	22.08 ^c \pm 2.62	11.93 ^b \pm 2.26
'F' Value	19.36**	7.14**	1.27	0.81	23.02**	7.86**

Means bearing different superscripts between columns differ significantly (*- $P \leq 0.05$; **- $P \leq 0.01$)

DPPH Radical Scavenging Activity

DPPH radical scavenging activity reflects the antioxidant potential of the chicken nuggets incorporated with *S. tenerrimum*. The antioxidant potential is attributed to the presence of phytochemicals in seaweed especially in the brown seaweed which has been documented to produce higher antioxidant activity than red and green seaweeds invitro (Nightingale, 2007) and hence in the product. The control nuggets were also recorded to contain antioxidant activity as observed by the higher percentage of inhibition of the DPPH radical (85.86 \pm 0.76 %). This could be attributed to the antioxidant potential of Kodo millet which has been used @ 7 per cent level in chicken nuggets. Higher activity observed in treatment nuggets reflects synergistic effect of the antioxidant potential of millet and seaweed due to the presence of phytochemicals which contribute to the functional benefit of antioxidant activity in the chicken nuggets. Improvement in the antioxidant quality in fish jerky incorporated with *S. wightii* was reported

by Hanjabam *et al.* (2016). Increasing inclusion levels of *S.tenerrimum* (Fig. 1.) has imparted increasing percentage of inhibition of DPPH radical activity (87.43% to 90.30 %), reflecting the potential possibility of being utilised for the production of functional chicken nuggets.

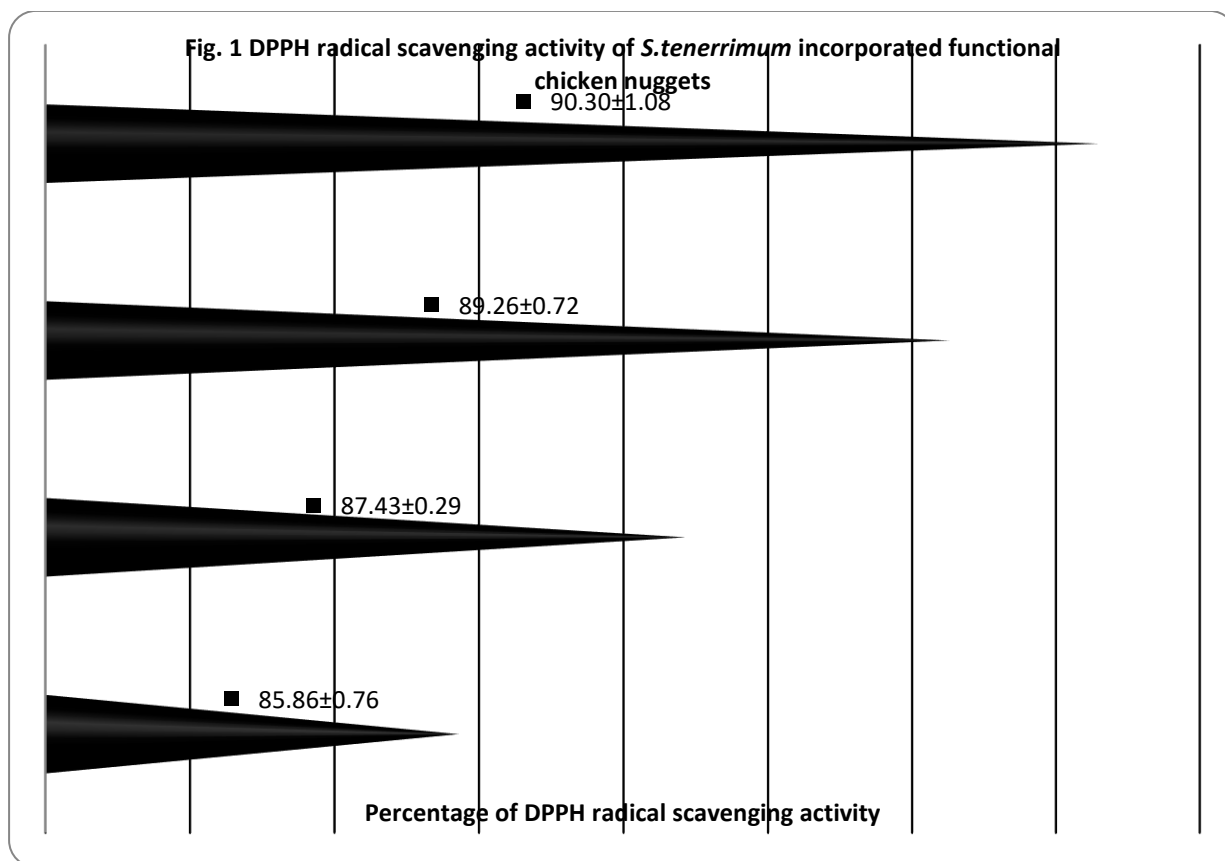


Figure 1: DPPH radical scavenging activity of *S. tenerrimum* incorporated chicken nuggets

Legend: C- Control Nuggets; T1- 0.25% *S. tenerrimum* incorporated functional chicken nuggets; T2- 0.5% *S. tenerrimum* incorporated functional chicken nuggets; T3- 0.75% *S. tenerrimum* incorporated functional chicken nuggets

Organoleptic Quality Characteristics

The results of organoleptic quality characteristics were presented in Table 6. Highly significant difference between control and treatment nuggets were observed for the organoleptic quality characteristics.

Table 6: Mean±SD of the organoleptic characteristics of *S. tenerrimum* incorporated functional chicken nuggets

Groups	Appearance	Flavour	Texture	Juiciness	Overall Acceptability
Control	8.38 ^c ± 0.36	8.34 ^{bc} ± 0.39	8.26 ^c ± 0.33	8.33 ^c ± 0.34	8.27 ^b ± 0.38
T1	7.98 ^{bc} ± 0.47	7.97 ^{bc} ± 0.41	7.75 ^{bc} ± 0.54	7.76 ^b ± 0.30	7.76 ^b ± 0.39
T2	7.71 ^b ± 0.54	7.70 ^b ± 0.49	7.51 ^b ± 0.49	7.58 ^b ± 0.31	7.84 ^b ± 0.50
T3	5.72 ^a ± 0.44	5.48 ^a ± 0.49	5.18 ^a ± 0.76	5.38 ^a ± 0.57	5.08 ^a ± 0.56
'F' Value	40.39**	49.34**	36.55**	64.63**	58.88**

Means bearing different superscripts between columns differ significantly (*- $P \leq 0.05$; **- $P \leq 0.01$)

However, decline in values were observed with increase in the inclusion levels of *S. tenerrimum* in millet-based chicken nuggets owing to the brown colour and seafood flavour that the seaweed imparts. The fact that there is statistical similarity between T1 and T2, after which the scores declined suggest that the product is acceptable till 0.5% incorporation (T2) of *S. tenerrimum* in chicken nuggets. Choi *et al.* (2015) and Jeon and Choi (2012) had also observed decline in appearance, flavour and overall acceptability scores in pork frankfurters and pork patties

incorporated with seaweed. Contrary results of higher flavour and overall acceptability scores in pork patties (Jeon and Choi, 2012) and chicken patties (Das *et al.*, 2013); improved tenderness, juiciness and overall acceptability in breakfast sausages (Kim *et al.*, 2010) in pork patties (Choi *et al.*, 2012) reveals the influence of the type of seaweed utilised in the development of the meat product.

Conclusion

Effective utilisation of the anti-oxidant potential of brown seaweed, *S. tenerrimum* in the development of functional chicken nuggets was achieved by designing the formulation with *S.tenerrimum* and studying its quality characteristics. The results of the product and quality characteristics and the invitro anti-oxidant activity suggests positive influence of seaweed with increasing concentrations. However, in the context of acceptance of the product with respect to the Indian palate, the inclusion level of 0.5% of *S. tenerrimum* was selected as the optimum level in the development of functional chicken nuggets.

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Conflict of Interests

There is no conflict of interest.

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