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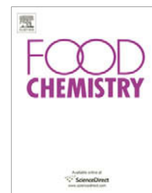

Edible Japanese seaweed, wakame (*Undaria pinnatifida*) as an ingredient in pasta: Chemical, functional and structural evaluation

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ABSTRACT

Wakame (*Undaria pinnatifida*) is edible seaweed rich in fucoxanthin; whilst, pasta is an important dish from nutritional and gastronomic point of view. Pasta was prepared with wakame as an ingredient at different levels. *In vitro* antioxidant properties, total phenolic content, fatty acid composition, fucoxanthin and fucosterol contents formed the major bio-functional characteristics analysed. Pasta with 10% wakame was acceptable sensorially. The total phenolic content varied between 0.10 and 0.94 mg gallic acid equivalents (GAE)/g, whilst total antioxidant activity varied from 0.16 to 2.14 mg ascorbic acid equivalents (AAE)/g, amongst different samples. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and superoxide radical scavenging activities of sensorily acceptable pasta were 7.71% and 4.56%, respectively. The sensorily acceptable pasta had a mild seaweed flavour with taste similar to control pasta, as assessed by panelists. The ratio of n-3 to n-6 fatty acid in seaweed incorporated pasta was 1:3.4 as compared to 1:15.2 in the control. Heat process involved in pasta preparation and cooking did not destroy fucoxanthin. Microstructure studies revealed the enhanced interaction between starch granules and protein matrix in pasta containing seaweeds up to 20%.

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1. Introduction

Marine environment is a major potential source of functional materials, including Omega-3 oils, essential minerals and vitamins, antioxidants, peptides and enzymes (Seafoodplus, 2008). Marine macro-algae or seaweeds are a part of staple diet from time immemorial in the orient as they are nutritionally rich materials (Dawczynski, Schubert, & Jahreis, 2007); but to a much lesser extent in the rest of the world. They are excellent source of vitamins, dietary fibres, minerals and proteins (Lee et al., 2008). In addition, products mainly hydrocolloids derived from seaweeds have been in use in cosmetics, pharmaceutical and food industries (Chandini, Ganesan, Suresh, & Bhaskar, 2008). The major seaweeds that are of dietary importance in several countries including Japan, China and Korea belong to the genus *Undaria* (commonly called as wakame), *Porphyra* (commonly referred as nori) and *Laminaria* (commonly known as kombu).

The total global seaweed production in the year 2005 was around 1.3 million tonnes by capture and 14.8 million tonnes by aquaculture (FAO, 2007). Increased research attention is being paid to marine macro-algae as sources of major bioactive compounds including carotenoids, fatty acids and phytosterols as they have been reported to possess several beneficial effects including antioxidant (Chandini, Ganesan, & Bhaskar, 2008; Nagai & Yukimoto, 2003), anticoagulant, antitumor and anti-cancerous properties (Lee et al., 2008). They have been paid much attention in a bid to develop new drugs and health foods. Brown seaweeds are known to contain more bioactive components than either green or red seaweeds (Seafood+, 2008). Some of the bioactive compounds identified in brown seaweeds include phylopheophylin, phlorotannins, fucoxanthin and various other metabolites (Hosakawa, Bhaskar, Sashima, & Miyashita, 2006).

Wakame (*Undaria pinnatifida*), one of the widely consumed brown seaweed is rich in fucoxanthin (Miyashita & Hosokawa, 2008; Shiratori et al., 2005). Fucoxanthin is a xanthophyll characteristic of brown seaweed and is the most abundant amongst aquatic carotenoids accounting for more than 10% of estimated total natural production of carotenoids (Hosakawa et al., 2006).

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Fucoxanthin and its metabolites have been reported to possess anti-oxidative, anti-cancerous, anti-obesity and anti-inflammatory (Miyashita & Hosokawa, 2008) properties. Apart from having important bioactive compounds wakame is also rich in protein (>15%) (Kunio & Takahisa, 2000). Several studies have reported that dietary ingestion of wakame decreases blood pressure in humans (Chandini, Ganesan, Suresh, et al., 2008). Furthermore, antioxidants from natural source as an ingredient to increase the quality and shelf-life of foods considerably enhances the consumer preference (Farag, El-Baroty, & Basuny, 2003).

Pasta is a staple food in many countries and pasta products are well accepted worldwide because of their low cost, ease of preparation, versatility, sensory attributes and long shelf-life (Bergman, Gualberto, & Weber, 1994). It is mainly used as an energy source due to its high content of carbohydrates. Pasta products have been fortified to enhance their nutritional properties with supplements from various high-protein sources, such as soy flours, soy isolates, milk and milk products, whey proteins, yeast protein concentrates and germinated pigeon pea (Torres, Frias, & Granito, 2007). Although, the nutritional properties of seaweeds are not completely known yet, they are usually estimated for their chemical composition alone (Chandini, Ganesan, Suresh, et al., 2008). More recently, Borneo and Aguirre (2008) studied the quality and consumer acceptance of pasta with amaranth plant (leaves) as a component.

We aimed to identify a food-based application for edible Japanese seaweed in order to make it popular amongst non-seaweed eaters. The idea was to scientifically evaluate and demonstrate a functional product in advance of its potential (possible) commercial exploitation. The primary focus of this work was to develop a product based on seaweed as an ingredient that will have functional compounds like fucoxanthin and fucosterol apart from good fatty acid and amino acid composition. Thus the present investigation evaluated the effect of different levels of brown seaweed, *Undaria pinnatifida* on the sensory, cooking, nutritional and bio-functional quality of pasta.

2. Materials and methods

2.1. Materials

T. Durum semolina was procured from the local market at Mysore (India). Commercial samples of Japanese seaweed (Wakame; *Undaria pinnatifida*) powder were procured from super market at Hakodate (Japan). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulphonic acid (ferrozine) and β-carotene were purchased from Sigma–Aldrich Chemie (Steinheim, Germany). Folin–Ciocatteau's phenol reagent, pyrogallol and potassium ferric cyanide were purchased from Merck (Mumbai, India); whilst, fucosterol was procured from Extra-synthase (Munich, Germany). Fucoxanthin (>99%) purity was prepared from wakame (*Undaria pinnatifida*) lipids as described previously (Maeda, Hosokawa, Sashima, Funayama, & Myashita, 2005). All other solvents and chemicals were of analytical grade.

2.2. Sample preparation

Commercial samples of semolina was analysed for moisture, ash, gluten and protein content according to the method of AACC (2000). Granulation test was carried out in Buhler plan sifter (Type MC 41 KS; Buhler GmbH, Braunschweig, Germany) using 670, 480, 340 and 193 micron sieves with 200 g sample. The over tailings of each sieve were weighed after running the sifter for 10 min. Proximate composition of commercial sample of wakame powder and prepared pasta were estimated as per methods outlined in AOAC (2000).

Calibration curves to determine concentrations of fucosterol and fucoxanthin in pasta sample were prepared using standard fucosterol and fucoxanthin. Amino acid and fatty acid composition were analysed only control and 10% seaweed incorporated pasta as sensory and bio-functional quality was higher in 10% seaweed incorporated pasta.

2.3. Pasta preparation

Semolina and seaweed blends were prepared, by replacement method, in the ratio of 100:0; 95:5.0; 90:10; 80:20 and 70:30 (semolina/wakame; w/w). Pasta dough (1000 g) was prepared and mixed for 7 min in a Hobart mixer (model N-50, Ontario, Canada) at 59 rpm. The dough was extruded using laboratory scale extruder (La Monferrina, Castell' Alfero–AT, Italy) fitted with dyes having perforation of 0.7 mm diameter. The extruded pasta was dried at 75 °C for 3 h in a hot air drier (Shrisat Electronics, Mumbai, India).

2.4. Quality characteristics of pasta

2.4.1. Cooking quality (cooking loss, cooked weight and pasta firmness)

To determine the cooking loss, 25 g of raw pasta was added to 250 ml of boiling water for 8 min. After cooking for 8 min, the material was drained for 5 min and volume of collected gruel (drained liquid) was measured. The gruel was stirred well and 20 ml of the gruel was pipetted into a petri plate and evaporated to dryness over a water bath (Scientific Lab Equipments, Chennai, India). Then the plate was transferred to a hot air oven (Memmert GmbH, Schwabach, Germany) for 16–18 h at 105 ± 2 °C and dried to constant mass (Indian Standards Institution [ISI]., 1993). Cooked weight was taken by weighing the drained pasta and expressed in grams. Pasta firmness was measured as described in Prabhasankar, Jyotsna, Indrani, and Venkateswara Rao (2007) by using a texture analyser (TA.HDi, Stable Microsystems, Surrey, UK). To measure the shear force, three cooked pasta strands were sheared at 90° angle. Shear was performed at a crosshead speed of 50-mm/min and load cell of 5 kg. The force required to shear the pasta was measured ($n = 4$).

2.4.2. Sensory characteristics

The panel consisting of semi-trained panelists ($n = 15$) who were regular and native eaters of wakame was employed for the sensory evaluation of pasta samples. The panelists were asked to assign scores for appearance (maximum of 10), strand quality (maximum of 10) and mouth feel (maximum of 10). The overall quality (maximum of 30) was computed by combining (30) scores of all the three attributes.

2.5. Microstructure

The cooked pasta samples were freeze-dried using Heto freeze dryer (DW3, Allerod, Denmark). Surface and cross section of freeze-dried samples were mounted on the specimen holder and sputter-coated with gold (2 min, 2 mbar). Finally, each sample was transferred to the microscope where it was observed at 15 kV and a vacuum of 9.75×10^{-5} Torr. A scanning electron microscope (Leo 435 VP, Leo Electronic Systems, Cambridge, UK) was used to scan the images.

2.6. Antioxidative properties of pasta

Aqueous extracts of pasta samples (3.0 g in case of raw; 9.0 g in case of cooked) were prepared by homogenising pasta in 30 ml of distilled water and filtered. The filtrates were made to 30 ml with distilled water. These extracts were used for determining various

in vitro antioxidant activities. All the methods including estimation of total phenolics, total antioxidant activity and other *in vitro* antioxidant assays have recently been detailed in our works (Chandini, Ganesan, & Bhaskar, 2008; Ganesan, Chandini, & Bhaskar, 2008).

2.6.1. Total phenolic content and total antioxidant activity

Briefly, 0.2 ml aliquot of sample was mixed with 4.0 ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. 0.2 ml of 50% Folin–Ciocalteau's phenol reagent was added, and the reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. After incubation, absorbance of all the sample solutions was measured at 720 nm using spectrophotometer (Shimadzu, Kyoto, Japan). Phenolic contents are expressed as gallic acid equivalents (GAE) per gram (GAE/g).

With regards to estimation of total antioxidant activities of pasta extracts, 0.2 ml of sample was mixed with 0.1 ml distilled water and 3.0 ml reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4.0 mM ammonium molybdate). Reaction mixture was incubated at 95 °C for 90 min under water bath. After incubation, absorbance of all the sample mixtures was measured at 695 nm. Total antioxidant activity is expressed as ascorbic acid equivalents (AAE) per gram (AAE/g).

2.6.2. DPPH radical scavenging activity

Briefly, 2.0 ml of 0.16 mM DPPH solution (in methanol) was added to the test tube containing 2.0 ml aliquot of sample (0.2 ml sample and 1.8 ml distilled water). The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The absorbance of all the sample solutions was measured at 517 nm. The scavenging effect (%) was calculated by using the formulae as mentioned in Chandini, Ganesan, and Bhaskar (2008). Sample blank and control samples were performed according to the method.

2.6.3. Superoxide radical scavenging activity

Briefly, 2.6 ml of 50 mM phosphate buffer (pH 8.2) were added to the test tube containing 0.3 ml of sample (0.2 ml sample and 0.1 ml distilled water). 0.09 ml freshly prepared pyrogallol (3 mM dissolved in 10 mM HCl) was added to the sample mixture. The inhibition rate of pyrogallol auto-oxidation was measured at 325 nm at every one minute interval for 10 min. Sample blank and control samples were performed according to the method. The scavenging activity (%) was calculated using the formula as mentioned in our earlier work (Chandini, Ganesan, & Bhaskar, 2008).

2.6.4. Metal chelating activity

Ferrous ion chelating activity was estimated according to the method of Decker and Welch (1990) with slight modification in the volume of sample and reagents. Briefly, 0.5 ml of distilled water and 0.125 ml ferrous chloride (0.5 mM) were added to the sample solution (0.2 ml sample and 0.3 ml distilled water). Absorbance (Abs) was measured at 550 nm (Abs 1). 0.125 ml ferrozine (2.5 mM) was added to the tubes containing above given reaction mixture and incubated at room temperature for 20 min. After incubation, absorbance was measured at same wavelength (Abs 2). The activity (%) was calculated according to the formula given by Kuda, Tsunekawa, Goto, and Araki (2005).

2.6.5. Reducing power

Briefly, 0.2 ml of pasta extract was mixed with 0.8 ml distilled water and 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1.0%). Reaction mixture was incubated at 50 °C for 20 min. After incubation, 2.5 ml of trichloroacetic acid (10%) was added and centrifuged ($650 \times g$) for 10 min. From the upper layer, 2.5 ml solution was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1%). Absorbance of all the sample solutions was measured at 700 nm. Increased absorbance is indicated increased reducing power.

2.7. Fucoxanthin and fucosterol contents

Lipids from different pasta samples were extracted by employing Bligh and Dyer (1959) using mixture of chloroform and methanol (2:1; v/v). Extracted lipids (~5 mg per ml) were subjected to reverse phase-HPLC analysis for determining fucosterol and fucoxanthin concentrations. HPLC was carried out on a Hitachi L-7000 system (Hitachi Ltd., Tokyo, Japan) equipped with an online analysis software (Hitachi HPLC system-5-manager; Model D-7000) and a photo-diode array (PDA) detector (Hitachi L-7455). The analysis employed a C30 column (Develosil C30 UG-5, 250×8.0 mm i.d., 5.0 μm particle size, Nomura Chem. Co., Aichi, Japan) protected with a guard column (10×4.0 mm i.d.) with the same stationary phase. The analysis carried out at 28 °C using mixture of methanol and acetonitrile (70:30, v/v) as the mobile phase at a flow rate of 1.0 ml/min. The eluent was detected at 450 nm and 210 nm for fucoxanthin and fucosterol, respectively. Concentrations of fucoxanthin and fucosterol were determined using the calibration curves prepared using standard fucoxanthin and fucosterol.

2.8. Amino acid and fatty acid composition

2.8.1. Amino acid composition and chemical score

Amino acid composition was determined using phenyl isothiocyanate (PITC) pre-column derivatization by employing Water's PicoTag Column and Workstation. Considering the contents of essential amino acids (EAA), chemical score of the protein hydrolysate was computed by employing the EAA in FAO/WHO standard protein. All the methods are described in Bhaskar, Benila, Radha, and Lalitha (2008). In brief the chemical score was calculated using the following relation –

$$\text{Chemical score} = \frac{\text{EAA in test protein}\,(\text{g } 100\text{ g}^{-1})}{\text{EAA in standard protein}\,(\text{g } 100\text{ g}^{-1})}$$

2.8.2. Fatty acid composition

Lipids were extracted from pasta samples as described elsewhere in the text. Pasta lipids were methylated as per the method of Prevot and Mordret (1976) to obtain fatty acid methyl esters (FAME). FAME obtained from different samples were subjected to gas–liquid chromatography (GC) analysis to determine their fatty acid composition. GC analysis was carried out on a Shimadzu GC-14B (Shimadzu Seisakusho, Kyoto, Japan) equipped with a flame-ionisation detector and a capillary column (Omegawax 320, 30 m $\times$ 0.32 mm i.d., Supelco, Bellefonte, PA). The column, injector and detector temperatures employed for the analysis were 200, 250 and 260 °C, respectively. Helium at a flow rate of 50 kPa was used as the carrier gas.

2.9. Statistical analysis

The experiments were carried out in four different batches. The means of all the parameters were examined for significance by analysis of variance (ANOVA) and in case of significance, mean

separation was accomplished by Duncan's multiple range test using STATISTICA software as detailed in Ganesan et al. (2008).

3. Results and discussion

3.1. Proximate composition, fucoxanthin and fucosterol contents

Semolina used for the studies had following characteristics on dry basis: ash 0.9%, dry gluten 10.0% and protein 11.6%. The particle size distribution test showed 67.85% of semolina retained on 32 W (678 μ) sieve, 25.20% on 45 W (480 μ), 6.50% on 60 W (340 μ) and 0.45% on 7XX (193 μ). Wakame (*U. pinnatifida*) powder used in the present study had 20.51% protein, 3.71% fat, 43.05% carbohydrate, 26.06% ash and 0.72% fibre content (all contents on dry weight basis). Dawczynski and co-workers (2007) have reported a similar protein (19.8%) and fat content (4.5%) in some edible seaweeds and their products. Proximate composition of different pasta samples is outlined in Table 1A. Replacing pasta ingredients with wakame powder considerably improved the protein and fat contents ($p < 0.05$). Likewise, increased levels of wakame powder significantly increased the ash and fibre contents of pasta ($p < 0.05$).

Control pasta had no fucoxanthin or fucosterol contents for obvious reasons. Increased levels of seaweed incorporations increased the fucosterol (0.51–2.55 mg/g) and fucoxanthin (0.02–0.23 mg/g) contents in seaweed pasta samples (Table 1). Fucoxanthin is the major bio-functional pigment of brown seaweeds and the content in various edible seaweeds including *U. pinnatifida* has been reviewed by Hosakawa et al. (2006). Further, HPLC analysis of cooked pasta samples revealed that both fucoxanthin and fucosterol were not affected by the processing steps involved in pasta making as well as the cooking step in pasta preparation. Hence, it can be concluded that fucoxanthin being an active and beneficial ingredient does not get destroyed due to processing steps involved or cooking steps thereafter.

3.2. Pasta quality

Cooking characteristics of different pasta samples are presented in Table 1B. Cooked weight increased with increased seaweed levels. This could be possibly due to hydration afforded by the hydrocolloids present in the seaweed powder. Weight gain upon cooking ranged from 2.34 (control) to 2.72 (30% wakame) grams per gram of uncooked pasta. However, cooking loss was highest (10%) in case of 30% seaweed incorporated pasta as compared to other samples.

Variations in the cooking loss have earlier been observed when pasta-like products incorporated with defatted soy, and wheat protein concentrate (Prabhasankar et al., 2007). Sensory analysis of different pasta samples revealed that pasta samples containing seaweed powder up to 10% had higher acceptance rating by the panelists. There was no significant difference ($p > 0.05$) in sensory scores of control and 5% seaweed containing pasta as well as 5% and 10% seaweed containing pasta. However, the other samples where seaweed content increased beyond 10%, sensory scores reduced significantly ($p < 0.05$) compared to control. In case of pasta samples containing 20% and 30% wakame powder, panelists complained of saltiness as well as they feel similar to that of eating wakame. Also, 30% seaweed containing pasta was not preferred sensorily as indicated by the lower scores for appearance and mouth feel. Hence, it was concluded that wakame powder up to 10% as better both in terms of sensory characteristics and additional nutritional value. Thus amino acid and fatty acid compositions were done only in case of 10% seaweed containing pasta and compared to that of control pasta.

3.3. Total phenolic content and total antioxidant activity

Phenolic compounds are commonly found in plants and have been reported to possess biological activity like antioxidant activity (Ganesan et al., 2008). Aqueous extracts of uncooked and cooked pasta containing seaweed exhibited higher phenolic content (Table 2A) as compared to control ($p < 0.05$). Our earlier research reported that extracts from brown and red seaweeds had considerable phenolic contents (Chandini, Ganesan & Bhaskar, 2008; Ganesan et al., 2008). However, the increase was not significant between pasta with 10%, 20% and 30% wakame powder. In case of cooked pasta, control samples had negligible phenol content (0.09 mg GAE/g). This could be due to leaching of phenolic compounds into the cooking medium and indicates the fact that its gruel had considerably higher phenolic contents (0.27 mg GAE/g). However, retention of

Table 1
(A) Proximate composition of different pasta samples along with their fucoxanthin and fucosterol contents; (B) Sensory and instrumental quality characteristics of different pasta.

Parameter studied^{y}	Control	W-5^{p}	W-10^{q}	W-20^{r}	W-30^{s}
(A) Composition of different pasta samples					
Moisture, %	8.78 ± 0.14^{a}	7.07 ± 0.14^{b}	10.82 ± 0.21^{c}	10.72 ± 0.49^{c}	9.86 ± 0.32^{d}
Protein, %	18.70 ± 0.28^{a}	18.92 ± 0.37^{a}	19.48 ± 0.19^{b}	$20.26 \pm 0.61^{b,c}$	21.68 ± 1.08^{c}
Fat, %	0.59 ± 0.01^{a}	0.65 ± 0.03^{b}	0.97 ± 0.01^{c}	1.94 ± 0.06^{d}	2.72 ± 0.13^{e}
Ash, %	0.89 ± 0.03^{a}	1.39 ± 0.03^{b}	1.69 ± 0.01^{c}	3.33 ± 0.10^{d}	4.82 ± 0.24^{e}
Carbohydrate, %	79.77 ± 1.20^{a}	78.92 ± 1.58^{a}	77.67 ± 0.78^{a}	74.20 ± 2.23^{b}	70.39 ± 3.52^{c}
Crude fibre, %*	0.05^{a}	0.12^{b}	0.19^{c}	0.27^{d}	0.39^{e}
Fucoxanthin, mg/g^{z}	nd	0.02^{a}	0.04^{b}	0.13^{c}	0.23^{d}
Fucosterol, mg/g^{z}	nd	0.51^{a}	1.25^{c}	1.66^{b}	2.55^{d}
(B) Sensory and instrumental quality characteristics					
Level of wakame, %	0.0	5.0	10.0	20.0	30.0
Cooked weight, g/25 g	58.6 ± 0.40^{a}	62.1 ± 1.30^{b}	62.4 ± 1.12^{b}	66.3 ± 0.83^{c}	67.9 ± 1.36^{c}
Cooking loss, g/100 g	5.1 ± 0.10^{a}	5.6 ± 0.17^{b}	5.8 ± 0.13^{b}	8.0 ± 0.16^{c}	10.0 ± 0.27^{d}
Shear force, g	69 ± 1.00^{a}	74 ± 0.50^{a}	78 ± 1.50^{b}	74 ± 1.00^{b}	72 ± 1.00^{a}
Appearance, max. 10	9.7 ± 0.51^{a}	$9.4 \pm 0.42^{a,b}$	9.0 ± 0.35^{b}	6.8 ± 0.43^{c}	5.6 ± 0.55^{d}
Strand quality, max. 10	9.6 ± 0.46^{a}	$9.1 \pm 0.60^{a,b}$	9.0 ± 0.61^{b}	7.3 ± 0.43^{c}	6.44 ± 0.58^{d}
Mouth feel, max. 10	9.4 ± 0.46^{a}	$9.1 \pm 0.22^{a,b}$	8.83 ± 0.25^{b}	6.44 ± 0.58^{c}	5.2 ± 0.35^{d}
Overall quality, max. 30	28.7 ± 1.28^{a}	$27.6 \pm 0.93^{a,b}$	26.8 ± 1.06^{b}	20.6 ± 1.24^{c}	17.2 ± 1.37^{d}

p,q,r,s: Pasta samples incorporated with 5%, 10%, 20% and 30% Wakame powder.
^{y}: All on dry weight basis except moisture; g/100 g dry weight.
^{z}: Standard error is less than 0.01, hence not expressed; content in uncooked pasta samples.
nd: Not detected.
a,b,c,d,e: Row wise values with different superscripts are significantly different ($p < 0.05$).

Table 2
Total phenolic content, total antioxidant activity and various *in vitro* antioxidant activities (DPPH-radical scavenging, superoxide radical scavenging, metal chelating and reducing power) of different pasta samples.

Sample	Raw	Cooked	Gruel
(A) Total phenolic content, mg gallic acid equivalents/g (GAE/g)			
Control	0.56 ± 0.015[a]	0.09 ± 0.002[a]	0.27 ± 0.010[a]
W-5[p]	0.88 ± 0.012[b]	0.17 ± 0.010[b]	0.27 ± 0.042[a]
W-10[q]	0.94 ± 0.006[c]	0.24 ± 0.022[c]	0.28 ± 0.033[a]
W-20[r]	1.07 ± 0.090[c]	0.29 ± 0.015[c]	0.29 ± 0.086[a]
W-30[s]	0.90 ± 0.052[bc]	0.27 ± 0.061[c]	0.30 ± 0.017[a]
(B) Total antioxidant activity, mg ascorbic acid equivalents/g (AAE/g)			
Control	0.61 ± 0.072[a]	0.16 ± 0.020[a]	0.30 ± 0.030[a]
W-5	1.33 ± 0.015[b]	0.29 ± 0.024[b]	0.75 ± 0.034[b]
W-10	2.14 ± 0.082[c]	0.88 ± 0.015[c]	0.83 ± 0.092[b]
W-20	2.82 ± 0.052[d]	0.69 ± 0.051[d]	1.10 ± 0.042[c]
W-30	2.68 ± 0.042[d]	0.43 ± 0.036[e]	1.16 ± 0.097[c]
(C) DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, %			
Control	6.83 ± 0.59[a]	5.72 ± 0.81[a]	8.67 ± 0.68[a]
W-5[p]	7.60 ± 0.17[b]	5.97 ± 0.16[b]	8.68 ± 0.89[a]
W-10[q]	8.31 ± 0.43[b]	7.71 ± 0.82[c]	8.25 ± 0.92[a]
W-20[r]	8.66 ± 0.44[c]	7.69 ± 0.68[c]	10.60 ± 1.07[b]
W-30[s]	9.79 ± 0.82[c]	6.95 ± 0.52[d]	9.78 ± 0.57[b]
(D) Superoxide radical scavenging activity, %			
Control	7.79 ± 1.00[a]	3.76 ± 0.60[a]	nd
W-5[p]	9.29 ± 0.85[b]	3.87 ± 0.30[a]	nd
W-10[q]	33.77 ± 1.23[c]	4.56 ± 0.56[a]	nd
W-20[r]	33.17 ± 1.67[c]	5.33 ± 0.71[b]	nd
W-30[s]	34.31 ± 1.15[c]	5.71 ± 0.35[b]	0.74 ± 0.28
(E) Metal chelating activity, %			
Control	1.49 ± 0.21[a]	9.72 ± 0.64[a]	1.88 ± 0.24[a]
W-5[p]	1.65 ± 0.13[a]	15.88 ± 1.12[b]	1.92 ± 0.31[a]
W-10[q]	2.58 ± 0.42[b]	29.27 ± 2.13[c]	7.72 ± 0.56[b]
W-20[r]	4.70 ± 0.65[c]	27.91 ± 1.67[c]	5.93 ± 0.42[c]
W-30[s]	5.36 ± 0.87[d]	28.32 ± 2.03[c]	6.27 ± 0.64[c]
(F) Reducing power			
Control	0.032 ± 0.002[a]	0.045 ± 0.002[a]	0.039 ± 0.004[a]
W-5[p]	0.030 ± 0.003[a]	0.058 ± 0.008[b]	0.042 ± 0.005[b]
W-10[q]	0.042 ± 0.001[b]	0.063 ± 0.003[b]	0.064 ± 0.002[c]
W-20[r]	0.048 ± 0.002[b]	0.059 ± 0.001[b]	0.051 ± 0.002[d]
W-30[s]	0.040 ± 0.002[b]	0.044 ± 0.003[a,c]	0.052 ± 0.003[d]

p,q,r,s: Pasta samples incorporated with 5%, 10%, 20% and 30% Wakame powder.
nd: Not detected.
a,b,c,d: Column wise values with different superscripts are significantly different ($p < 0.05$).

phenolic content was significantly higher in seaweed containing pasta as compared to control samples ($p < 0.05$). This clearly indicates that incorporation of seaweed results in retaining phenolic compounds in the product upon cooking.

Uncooked seaweed pasta exhibited higher total antioxidant content ($p < 0.05$) than the control pasta (Table 2B). With increased level of seaweed incorporation, total antioxidant content increased from 1.33 to 2.82 mg AAE/ g of pasta. Our earlier study on antioxidant properties of brown seaweed showed that total antioxidant activity of aqueous fraction of *Sargassum marginatum* was 1.09 mg AAE/g of extract (Chandini, Ganesan, & Bhaskar, 2008). Like phenolic content, increase was not significant beyond seaweed levels of 10% ($p > 0.05$). Total antioxidant activity decreased with seaweed levels beyond 10% in case of cooked pasta. This could possibly be due to leaching of materials upon cooking into the aqueous medium, as can be noticed in the increased levels of total antioxidant activity in case of gruels (Table 2B).

3.4. In vitro antioxidative properties

DPPH has been used extensively as a free radical to evaluate reducing substances and is a useful reagent for investigating *in vitro* free radical scavenging activities of compounds. Uncooked samples of seaweed incorporated pasta exhibited higher radical scavenging activity as compared to control. Increased levels of seaweed incorporation increased the radical scavenging activity from 6.83% to 9.79% (Table 2C). It has been earlier reported that some carbohydrate and protein enzyme hydrolysates of *Sargassum* had DPPH radical scavenging activity (Heo, Park, Lee, & Joen, 2005; Park, Shahidi, & Jeon, 2004). The heat degraded compounds from carbohydrate or protein that might have leached into the cooking medium could be responsible for increased activity noticed in case of gruels.

Superoxide scavenging activity of uncooked control pasta was 7.79%, which increased drastically to 33.77% in pasta incorporated with 10% seaweed level and beyond which the increase was not significant ($p > 0.05$). Nagai and Yukimoto (2003) recorded a significant superoxide anion scavenging activity for a beverage made from brown seaweed, *Hizikia fusiformis*, and suggested that studies on seaweeds concerning superoxide anion scavenging activity are of increasing interest due to the harmful effect of superoxide. On cooking the retention of superoxide radical scavenging activity of seaweed incorporated pasta were relatively higher as compared to control. No scavenging activity was noticed in gruels of control pasta and pasta with seaweed levels up to 20%. In general increase in antioxidant activities observed in seaweed pasta can be attributed radical scavenging activities of seaweed itself (Park et al., 2004).

Metal (ferrous ion) chelating activity of uncooked pasta increased from 1.49% to 5.36% with increasing levels (0–30%)of seaweed and increase in the content was significantly different between the samples ($p < 0.05$) (Table 2D). Aqueous fraction obtained from brown seaweeds has been reported to exhibit higher ferrous ion chelating activity than ethanol extracts (Kuda et al., 2005). However, on cooking metal chelating activity increased with increasing seaweed levels, but incorporation of seaweed beyond 10% was not significant ($p > 0.05$). Increased metal chelating activity could possibly be due to heat degraded products of carbohydrates or heat activation of compounds responsible for metal chelation. Also, a better network formation between gluten and starch granules as affected by incorporation seaweed, as evidenced from scanning electron micrographs of cooked pasta samples (Fig. 1) could also be one of the reasons.

Generally, reducing power gives a general view of reductones present in the sample (Ganesan et al., 2008) that are reported to be terminators of free radical chain reaction. Uncooked seaweed incorporated pasta samples exhibited marginal increase in reducing power as compared to control pasta. Reducing power increased with increasing seaweed content up to 20% in the case of uncooked pasta and up to 10% in the case of cooked pasta (Table 2E), although increase was not significant in case of seaweed containing pasta samples ($p > 0.05$). Kuda et al. (2005) have earlier reported increase in reducing power with increasing concentrations of different seaweed extracts.

3.5. Amino acid composition and chemical scores

Amino acid composition of 10% seaweed and control pasta samples is shown in Table 3. It can be seen that methionine, isoleucine, lysine, threonine and valine are the limiting amino acids, in that order, in case of both control and seaweed containing pasta samples. However, incorporation of wakame powder resulted in considerable improvements in the concentration of threonine, isoleucine, lysine and methionine contents and their chemical scores in pasta. This could be due to the higher concentration of these essential amino acids present in *U. Pinnatifida* (Dawczynski et al., 2007). Thus it can be concluded that incorporation of seaweeds not only improves the antioxidant properties and cooking characteristics of pasta but also aids in improving the amino acid pattern of pasta.

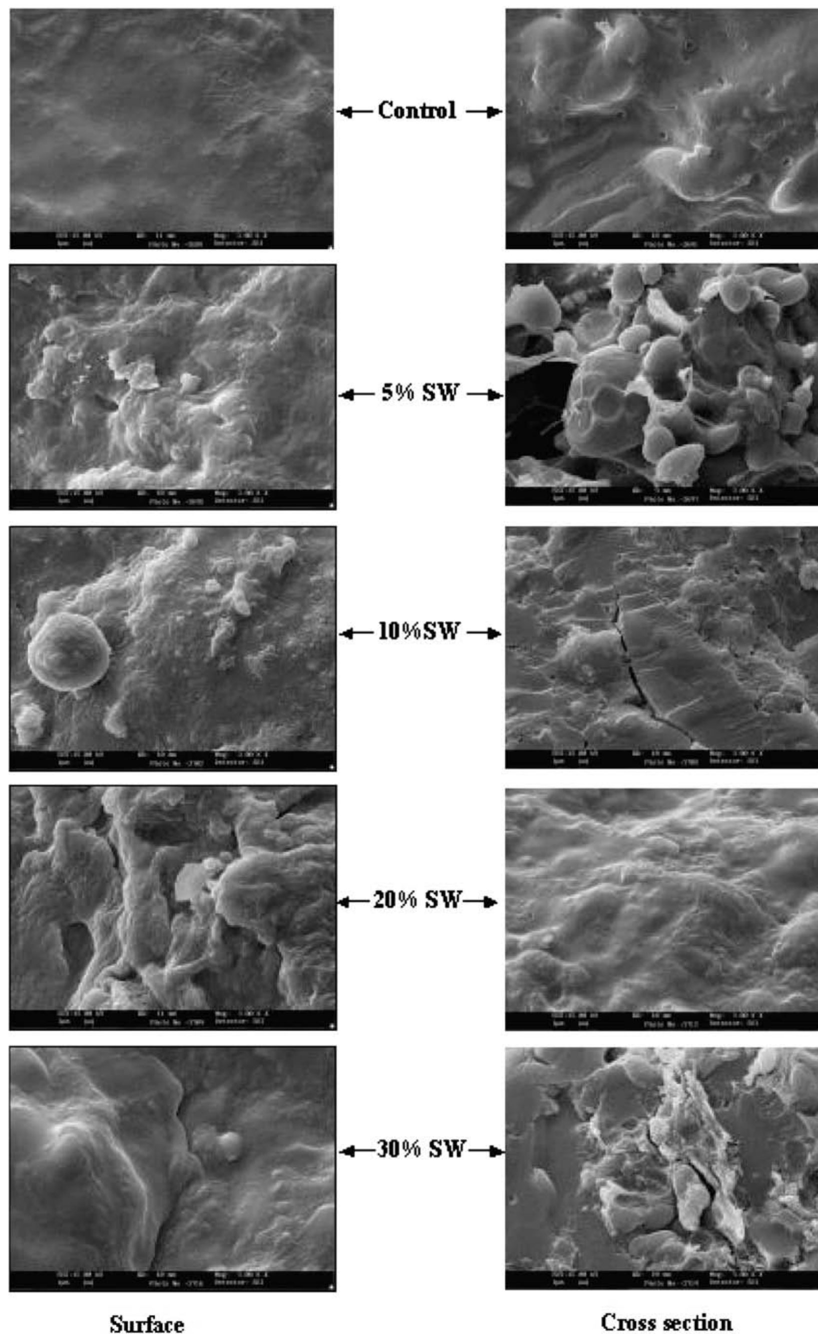

Fig. 1. Scanning electron micrographs of (a) surface and (b) cross sections of cooked (and lyophilised) pasta samples at 3000X indicating structural changes.

3.6. Fatty acid (% of total) composition

Fatty acid composition of control pasta samples indicates a skewed ratio of n-3 and n-6 fatty acids (Table 4) at 1:15.2 (w/w). However, for a diet to be healthy, it should have a balanced ratio between n-3 and n-6 fatty acids, which is usually 1:2 to 1:4 (w/w) (Gebauer, Harris, Kris-Etherton, & Etherton, 2004). Incorporation of wakame led to increased levels of n-3 fatty acids finally bringing down the ratio of n-3 to n-6 fatty acids from 1:15.2 to 1:3.4. Linoleic acid (18:2 n-6) was found to be the main fatty acid in case of both control as well as seaweed pasta. The lipids of most algae varieties consisted mainly of PUFA and highest Linoleic acid was observed in *U. pinnatifida* with 7% of total FAME (Dawczynski et al., 2007). Skewed ratio of n-3 to n-6 fatty acids is mainly due to the fact that control pasta did not have many long chain n-3 fatty acids; whilst, seaweed incorporated pasta contained considerable quantities of long chain n-3 polyunsaturated fatty acids in the form of octadecatetraenoic acid (18:4 n-3), eicosapentaenoic acid (20:5 n-3) and eicosatetraenoic acid (20:4 n-3). Earlier reports have emphasised the intake of food rich in n-3 long chain-PUFA can have a positive influence on the composition of blood lipids and prevention of arteriosclerosis (Gebauer et al., 2004).

3.7. Effect of pasta preparation on fucoxanthin and fucosterol

HPLC analysis of seaweed incorporated pasta samples clearly indicated fucoxanthin to be the major pigment (Table 1A) apart from fucoxanthin isomers and β-carotene (data not shown). The

Table 3
Amino acid composition (g/100 g protein) of control and wakame incorporated pasta along with their chemical scores.

Amino acids	Test protein[a]		Reference protein[b]	Chemical score[c]	
	Control	W-10[q]		Control	W-10[q]
(a) Essential amino acids					
Histidine	2.31	2.29	2.00	1.16	1.15
Isoleucine	3.59	3.92	4.00	0.90	0.98[y]
Leucine	7.98	8.15	7.00	1.14	1.16
Lysine	3.22	4.04	5.50	0.59	0.73[y]
Methionine	0.53	0.96	3.50	0.15	0.27[y]
Phenylalanine	5.15	4.96	4.29[d]	1.78	1.69
Tyrosine	2.48	2.30	–	–	–
Threonine	3.04	3.92	4.00	0.76	0.98[y]
Tryptophan	–	–	1.21	–	–
Arginine	5.21	5.15	5.00	1.04	1.03
Valine	4.53	4.96	5.42	0.84	0.92[y]
(b) Non-essential amino acids					
Asparagine/Aspartate	4.56	6.15			
Glutamine/Glutamate	32.29	29.45			
Serine	4.76	5.10			
Glycine	3.92	4.23			
Alanine	3.31	4.54			
Proline/ Hydroxyproline	12.24	9.26			
Cystine	0.88	0.62			

[a] Average of duplicates.
[b] Amino acid composition of reference protein as per FAO/WHO (Bhaskar et al., 2008).
[c] Chemical scores computed described in Bhaskar et al. (2008).
[d] Sum of tyrosine and phenylalanine.
[y] Considerable improvement in content and chemical score as compared to control.
[q] Pasta incorporated with 10% wakame powder.

Table 4
Fatty acid (% of total) composition of control and seaweed incorporated pasta.

Fatty acid	Control	W-10[q]
14:0	0.15	0.45
15:0	0.13	0.12
16:0	17.54	16.31
17:0	0.11	0.10
18:0	1.64	1.37
20:0	0.17	0.19
16:1n-7	0.15	0.22
18:1n-9	17.65	15.01
18:1n-7	0.85	0.69
18:2n-6	55.52	45.77
20:2n-6	0.10	0.10
16:3n-4	0.10	0.10
18:3n-6	0.18	0.21
18:3n-3	3.68	5.15
20:3n-6	–	0.08
20:3n-3	–	0.04
18:4n-3	–	5.91
20:4n-6	–	2.44
20:4n-3	–	0.11
20:5n-3	–	3.06
Others	2.03	2.57
SFA[x]	19.74	18.54
MUFA[y]	18.65	15.92
PUFA[z]	59.58	62.97
n-3 to n-6	1: 15.2	1: 3.4

[q] Pasta incorporated with 10% wakame powder.
[x] Saturated fatty acids.
[y] Monounsaturated fatty acids.
[z] Polyunsaturated fatty acids.

fact that the reduction in content of fucoxanthin and fucosterol was less than 10% (data not shown) upon cooking indicates the stability of fucoxanthin/fucosterol when present in the gluten network. This might be one of the reasons that the retention of higher bio-functional properties after cooking in seaweed incorporated pasta as compared to control. To our knowledge this is the first report regarding the stability of fucoxanthin in a food system.

3.8. Microstructure

Variation in the network of starch granules and protein matrix were observed in the micrographs of uncooked and cooked pasta samples (Fig. 1). The formation of gluten matrix is evident in case cross section micrographs of pasta samples containing 10% and 20% wakame powder. Beyond 20% level of seaweed, network again seems to be breaking which could possibly be the reason for not retaining compounds. This could be due to gumming activity of seaweed with gluten matrix. It has been reported that alginate forms a stable complex with starch, which in turn inhibits rupture on gelatinization, and reduced starch loss on subsequent cooking (Chawan, Merritt, & Matuszak, 1995). For good quality pastas, the thin film of protein network has to be formed and enveloping entire gelatinized starch granules is crucial in determining the cooking quality of pasta products (Jyotsna, Prabhasankar, Indrani, & Venkateswara Rao, 2004). In the present study, it is more evident from the surface micrographs of pastas that the incorporation of seaweed enhances this network formation up to the 20% level incorporation of seaweed. This was also supported by cross section micrographs of cooked pasta samples. The honeycomb like structure in which gelatinized starch granules entrapped in the gluten matrix was observed in the case of control. This network strength was enhanced in the case of pasta with seaweed up to 20%. Jyotsna et al. (2004) studied the effect of additives on the microstructure of vermicelli. They found the honeycomb network formed between protein and starch granules of cooked vermicelli has been affected by addition of additives. Similarly, Prabhasankar et al. (2007) studied the effect of addition of animal protein and additives on the microstructure of vermicelli. They found the gluten network (honey comb structure) has been affected by addition of whey protein concentrate (WPC). However, the microstructure structure of WPC incorporated vermicelli has been improved by addition of additives. The present SEM studies indicates that addition of edible seaweed (wakame) up to 20% level enhances the interaction between starch granules and protein matrix which resulted in improved quality pasta.

4. Conclusions

Edible seaweed, Wakame (*U. pinnatifida*) can be incorporated up to 20% as an ingredient of pasta was found to be sensorially acceptable with better bio-functional properties. But, the sensory analysis clearly indicates that pasta with 10% wakame as its ingredient had better quality score than 20% level. Incorporation of seaweed in the pasta system is not only result in value addition in terms of improved amino acid and fatty acid profile, but also increase the nutritional value of the pasta due to higher content of bio-functional components such as fucoxanthin and fucosterol. Fucoxanthin is not affected by the rigorousness of pasta making process as well as cooking step involved thereafter, points to one of the means by which fucoxanthin can be dietarily administered. It also opens up a new line of research where fucoxanthin can be made more stable if incorporated with some other macromolecules like protein, carbohydrate or lipids.

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