

Indian Journal of Geo Marine Sciences Vol. 51 (01), January 2022, pp. 18-25



Studies on nutritional composition of three colour forms of *Kappaphycus alvarezii* (Doty) Doty

K Suresh Kumar^{*,a}, Sushma Kumari^a & P V Subba Rao^b ^aDepartment of Botany, University of Allahabad, Allahabad – 211 002, India ^aAquaculture Foundation of India, Madurai – 625 003, Tamil Nadu, India *[Email: ksuresh2779@allduniv.ac.in] *Received 14 January 2021; revised 12 January 2022*

Biochemical composition of seaweeds is known to vary with species and environment. *Kappaphycus alvarezii* is an important commercial source of carrageenans (gel-forming and viscosifying polysaccharides). The nutritional and mineral composition of three (brown, green and pale yellow) colour forms of edible seaweed *K. alvarezii* is investigated in the current study. The brown colour form of this seaweed contained highest ash content of $(25.99\pm0.22 \%)$, crude fibre $(21.0\pm0.68 \%)$, and protein $(8.92\pm0.41 \%)$, while, the green form had highest carbohydrate $(22.0\pm0.00 \%)$ and sulphate $(5.89\pm0.00 \%)$ content. On the other hand, the pale yellow colour form had highest lipid content $(0.72\pm0.00 \%)$. Amongst the 17 minerals analyzed, highest total mineral macro-elements (*viz.* Na, K, Ca and Mg) were recorded in the brown form $(18.8\pm0.71 g/100 g d wt)$ followed by the pale yellow $(17.3\pm1.07 g/100 g d wt)$ and green colour form $(11.9\pm1.22 g/100 g d wt)$. The micro-elements varied within the three colour forms; however, the brown colour form had maximum micro-element content (P, Cd, Pb, As, Hg, Cr, Zn, Cu, Fe, Mn, Co, Mo and Ni; 19.96\pm0.24mg/100 g d wt), followed by green $(8.6\pm0.64 \text{ mg} / 100 g d wt)$ and pale yellow form $(7.15\pm0.58mg / 100 g d wt)$. On the basis of this study, it could be said that these three colour forms (*i.e.* brown, green and pale yellow) of *Kappaphycus alvarezii* could be utilized as a condiment in the omnivorous diet; however, a daily intake of 5.68 g d wt is recommended in case of the brown form, while in case of the green and pale yellow form a maximum daily intake of 9.55 and 10.5 g d wt, respectively is recommended. The study also reveals that the three colour-forms differed in their nutritional and mineral composition.

[Keywords: Colour forms, Composition, Kappaphycus alvarezii, Minerals, Nutritional profiling, Protein, Seaweed]

Introduction

Seaweeds owing to their low caloric index and high protein, carbohydrate, lipid, vitamin, and mineral (macro and micro elements) content, are considered as healthy food. They are rich in antioxidant molecules, polyphenols and other bioactive compounds¹⁻³. Apart from being used as food or fodder traditionally, they have been used for versatile applications (e.g. fertilizer and medicinal uses). Buschmann *et al.*⁴ stated the resourceful utilization of seaweeds in various forms *viz*. fresh, dried, powder or flakes, salted, canned, liquid extracts or as prepared foods; in addition to direct human consumption, processed seaweeds could be used as food additives and in nutraceuticals, biofuels, cosmetics and medicines.

Out of the 250 commercially utilized seaweed species, approximately 150 are consumed as food by humans⁵. People in Japan, China and Korea, chiefly consume seaweed in their diet. On the other hand, in Western and European countries, seaweeds are considered as a source of polysaccharides (agar, alginates and carrageenans) for food and

pharmaceutical industry. An intense upsurge in the world's population necessitates growth in food and feed production worldwide. The increasing demand of seaweeds in the market worldwide has compelled commercial cultivation of several seaweeds. Seaweed cultivation and collection of naturally occurring seaweed has turned out to be a remarkable source of livelihood^{6,7} especially for the coastal dwellers.

Often, stable colour mutants are considered genetic markers to investigate algal life histories, particularly mating systems⁸. The occurrence of various colour forms (among a natural population) generally depends on mutation rate, however, the maintenance of polymorphism depends on their relative fitness. According to Guimaraes *et al.*⁸, colour mutants (green, brown, purple, yellow, orange, and pink) are relatively wide spread in Rhodophyta; they have been observed in > 11 species. Nevertheless, reports suggest that green is the most frequently observed colour form⁹.

There are very few reports comparing the composition of various forms of the same seaweed. For example, Pereira *et al.*¹⁰ evaluated seasonal

variation in the pigment composition of three colour forms (red, green and brown variants) of *Gracilaria domingensis* (Gracilariales, Rhodophyta). Nonetheless, comparison of their nutritional and mineral content has been less thought about. The red edible seaweed, *K. alvarezii*, exhibits brown, green and red/ pale yellow colour forms¹¹. The feasibility of commercial cultivation of this exotic species, particularly of its colour forms, with socio-economic and environmental perspective has been evaluated by Hayashi *et al.*¹². The aim of this study is to evaluate and compare the nutritional profiles of three colour forms of *K. alvarezii i.e.* the conventional brown colour form, as well as, the green and the pale yellow forms cultivated on Northwest coast of India.

Materials and Methods

Collection of samples

Three colour forms of *K. alvarezii viz.* brown, green and pale yellow (Fig. 1) were collected from a cultivation site at Port Okha ($22^{\circ}28.528$ ' N; 069° 04.322' E), Northwest coast of India. The samples were thoroughly washed first with seawater and then with fresh water, followed by oven drying at 60 °C. The dried samples were ground (< 1 mm) and stored at room temperature in airtight plastic containers for further use.

Biochemical analysis

In this study, the nitrogen content of the dried seaweed samples was quantified by the Kjeldahl method¹³. This method quantitatively determines the nitrogen contained in organic substances as well as the nitrogen contained in the inorganic compounds ammonia and ammonium (NH_3/NH_4^+). For this, dried seaweed samples were digested with sulfuric acid (with 96 % H₂SO₄; initially for 75 min at 420 °C, and thereafter again for 75 min at 370 °C). Subsequently, the mixture was distilled with boric acid solution



Fig. 1 — Three colour forms of *K. alvarezii*: (a) Brown, (b) Green, and (c) Pale Yellow

(2 %) and titrated with 0.1 M HCl. This liquid was fed into Kjeldahl instrument KEL PLUS-KES 20L wherein, the digestion unit was attached to a KEL PLUS-CLASSIC DX Distillation unit (M/s PELICAN Equipments, Chennai, India). The instrument estimated the nitrogen content of each of the samples, which were later multiplied by a factor of 6.25 to obtain the value of crude protein content.

The total carbohydrate content was estimated using the phenol–sulphuric acid method¹⁴ (glucose was used as a standard). On the other hand, the crude lipids were extracted in asoxhlet extractor using 2:1 ratio of chloroform-methanol¹⁵.

The fibre content of the dried seaweed was determined using the standard method outlined by $AOCC^{16}$; here, acid hydrolysis was carried out with sulphuric acid (0.3 N H₂SO₄), while base hydrolysis was undertaken using sodium hydroxide (0.5 N NaOH). Cold extraction was carried out with acetone; thereafter, the sample was dried for 1 h (at 110 °C) or until it reached a constant weight in an oven. It was cooled in a desiccator, and weighed (W1). This sample was then placed in a muffle furnace (550 °C for 3 h), followed by which it was again cooled (in a desiccator) and reweighed (W₂). The crude fibre percentage was calculated following formula:

% *Crude fibre* = $(W_1 - W_2 / W_0) \times 100$

Where, W_0 was the initial weight of the dried seaweed which was 2 g.

In order to estimate the ash content, the seaweed samples were shade dried (at ambient room temperature) followed by oven drying (80 °C for 1 h); thereafter, they were ground to fine powder. One gram of powdered sample was accurately taken in a crucible and ashed in muffle furnace (550 °C for 6 h) to a constant weight. The ash obtained was quantified gravimetrically¹⁷. Further, the sulphate content was analyzed gravimetrically¹⁸.

Analysis of minerals by ICP-OES

One gram seaweed was ashed and cooled similar to the procedure mentioned above; the ash was then moistened by adding 10 drops of distilled water (Milli-Q) followed by careful dissolution in 3 ml HNO₃ (1:1 v/v). Thereafter the mixture was slowly heated on a water bath at 100 – 120 °C until the solution totally evaporated. The crucible was then returned to a muffle furnace, ashed for 1 h at 550 °C, and then cooled down to room temperature. Subsequently the ash was dissolved in 3 ml of 10 M HCl (1:1 v/v), and the solution was filtered through Millipore syringe filter (0.25 μ m) into 50 ml volumetric flask and 2 ml 0.1 N HCl was added to the filtrate and the final volume was made up to 50 ml using distilled water (Milli-Q)¹⁹. These samples were used for determination of mineral content (Na, K, Ca, Mg, P, Cd, Pb, As, Hg, Cr, Zn, Cu, Fe, Mn, Co, Mo and Ni) using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES Perkin-Elmer, Optima 2000). The analysis of each mineral was carried out in triplicate; thereafter, the mean and standard deviation were calculated. All the chemicals and solvents used for the experiments were of analytical grade.

Statistical analysis

A comparative study of the biochemical composition of the three colour forms (*i.e.* the brown, green and yellow) of *K. alvarezii* was undertaken in this study. Naturally occurring samples of the three colour forms were procured in three replicates (of each particular colour form) and analysed for their nutritional profile.

The mean and standard deviation and one–way analysis of variance (one–way ANOVA) was performed to confirm significant differences in response at p < 0.05.

Results and Discussion

The chemical composition of the three colour forms of K. alvarezii collected from Okha varied from each other (Table 1). Their protein content ranged from 8.13±0.17 to 8.92±0.41 %; this was notably higher than the report of Hurtado-Ponce¹¹, who grew three colour forms (brown, green and red) of the same species at different water depths in the Philippines. The protein content obtained herein was also higher than Codium reediae, Enteromorpha flexuosa, Dictyota sandvicensis, Ahnfeltiopsis concinna, Asparagopsis taxiformis. Eucheuma denticulatum, Gracilaria salicornia, Laurencia dotyi and Laurencia nidifica²⁰ which were 7, 7.9, 6.4, 5.7, 6.1, 4.9, 5.6, 2.7 and 3.2

Table 1 — Composition of the three colour forms of *K. alvarezii*. Values are expressed as mean \pm standard deviation. The different superscript letters are significantly different at *P* < 0.05 (ANOVA with least significant difference (LSD))

	Brown Green		Pale Yellow	
Ash	$25.99\pm0.22^{\text{a}}$	18.89 ± 0.17^{b}	$20.70\pm0.14^{\text{c}}$	
Crude fibre	$20.95\pm0.68^{\text{a}}$	20.49 ± 0.87^{a}	$16.77\pm0.68^{\text{b}}$	
Total soluble sugar	14.88 ± 0.07^{a}	21.95 ± 0^{b}	$21.80\pm0^{\rm c}$	
Protein	$8.92\pm0.41^{\text{a}}$	8.31 ± 1.09^{a}	8.13 ± 0.17^{a}	
Lipid	$0.66\pm0.01^{\text{a}}$	0.69 ± 0^{b}	$0.72\pm0^{\rm c}$	

g/100 g DW²⁰, respectively. The values obtained in this study are also higher than the protein content of Sargassum vulgare (4.59 – 9.97 g/100 g DW²¹; Ulva lactuca (7.06±0.06 g/100 g DW²²; Undaria pinnatifida $(7.5\pm1.9 \text{ g}/100 \text{ g DW}^{23}; \text{ and } Caulerpa veravelensis}$ $(7.77\pm0.59 \text{ g}/100 \text{ g DW})^{24}$. El Din & El-Sherif²⁵ reported the protein content of Caulerpa prolifera, Caulerpa racemosa, Codium bursa, Gracilaria verrucosa, Rhodvmenia ardissonei, C. spinosa, Dictyota Sargassum acinarium, dichotoma. Sargassum hornschuchii and Sargassum vulgare to be 3.86, 8.23, 5.35, 7.08, 4.5, 7.01, 8.15, 3.9, 6.05 and 6.15 g/100 g DW, respectively. These values are also lower than that obtained for the three colour forms of K. alvarezii in the present study and lesser than that reported by Mohammed *et al.*²⁶ for Wakame (10.2 g/100 g DW).

Seaweed polysaccharides prevent degenerative diseases including cardiovascular and type 2 diabetes. They increase the amount of feel-good chemicals in the brain, improve liver function, and stabilize blood sugar. In this study, the carbohydrate content of the green and yellow forms was at parity, while the brown recorded comparatively lower carbohydrate content (14.9±0.07 g/100 g DW). The amount of carbohydrate obtained in green and pale yellow forms was considerably higher than that reported for the Hawaiian edible red seaweeds Asparagopsis taxiformis (9.2 g/100 g DW), Gracilaria coronopifolia, Laurencia species²⁰. Ulva rigida, Ulva lactuca, Caulerpa racemosa, Sargassum filipendula, Hypnea japonica, C. prolifera, C. racemosa, C. bursa, H. tuna, U. petiolata, Udotea sp., G. corneum, R. ardissonei, D. dichotoma, S. Acinarium and S. vulgare; these are reported to contain 9.2, 6.40, 7.06, 3.98, 3.73, 4.28, 9.00, 6.93, 7.43, 7.40, 8.60, 8.00, 12.03, 5.00, 8.60, 13.67 and 14.30 g/100 g DW of carbohydrates, respectively^{25,27}.

The lipid content of seaweed (or marine macroalgae) generally range between 1 and 6 g/100 g DW²³. In the present investigation, the lipid content of the three colour forms ranged between 0.66 ± 0.01 to 0.72 ± 0.00 % (Table 1), which was comparable with *Corallina officinalis* $(0.3\pm0.2 \text{ g/100 g DW})^{28}$, *Sargassum polyschides* $(0.7\pm0.09 \text{ g/100 g DW})$, as well as *Hizikia* sp. $(0.7 \text{ g/100 g DW})^{29}$. The lipid content recorded in this study was higher than several vegetables like carrot, broccoli, red spinach, lettuce, tomato, red pumpkin, cabbage and red chilli³⁰.

Seaweeds are rich in dietary fibre as compared to most fruits, vegetables and terrestrial food stuff. Thus

21

consumption of seaweeds helps increase the intake of dietary fibre; this lowers the occurrence of some chronic diseases (diabetes, obesity, heart diseases and cancers). Intake of seaweed is known to protect and promote the growth of beneficial intestinal flora; it reduces the overall glycemic response, increases the stool volume and reduces the risk of colon cancer³⁰⁻³². The present study revealed that K. alvarezii was particularly rich in fibre. Its fibre content ranged from 16.8±0.68 to 21.0±0.68 % g/100 g DW (Table1); this was higher than that reported by Robledo & Freile-Pelegrin³³ who reported Sargassum vulgare (mean 7.40±1.61 g/100 g DW) and Gracilaria cervicornis (mean 5.65±0.74 g/100 g DW). On the other hand, Syad³⁴ reported the fibre content of Gelidiella acerosa and Sargassum wightii to be 13.45±1.07 and 17±1.19 g/100 g DW, respectively. The average fibre content of all three forms of K. alvarezii obtained in the present study, was higher than Ascophyllum nodosum, Laminaria digitata, Himanthalia elongate, Undaria pinnatifida, Porphyra umbilicalis, Palmaria palmate, Ulva sp., and *Enteromorpha* sp., which are reported to contain 8.8, 6.2, 9.8, 3.4, 3.8, 5.4, 3.8 and 4.9 % fibre, respectively³¹. However, whole food such as brown rice, prunes, porridge, lentils green/brown, cabbage, carrots, apples and bananas, are reported to contain 3.8, 2.4, 0.8, 8.9, 2.9, 2.6, 2.0 and 3.1 %, respectively³¹; these values are also much lower than the fibre content of K. alvarezii.

The ash content of a sample is generally known to reflect its mineral content. Mushollaeni³⁵ stated that the presence of ash content in seaweed indicated the presence of mineral salt in the sample. The current study on K. alvarezii, revealed highest amount of ash content in the brown form $(26.0\pm0.22 \%)$ followed by the pale yellow (20.7 ± 0.14 %) and green (18.9 ± 0.17 %) colour forms. The ash content of the three colour forms studied herein lie within the range reported for seaweeds, *i.e.* 8 to 40 % of algal dry weight³⁶⁻³⁸. The ash content of the pale yellow and green forms was similar to the red seaweeds Chondrus and Nori. Although the ash content of the brown colour form was similar to the other reports projecting the ash content of the similar form^{39,40}, it was less than other brown seaweeds viz. Fucus, Laminaria and $Wakame^{41}$. Nevertheless, the ash content of brown form recorded in this study was considerably higher than Hypnea japonica (22.1±0.72 g/100 g DW), Hypnea charoides (22.8±2.23 g/100 g DW), Ulva lactuca (21.3±2.78 g/100 g DW), Hypnea pannosa

(15.3 g/100 g DW), Ulva lactuca (24.6 g/100 g DW), Ulva pertusa (24.7 g/ 100 g DW), Ulva lactuca (11.0 \pm 0.1 g/100 g DW), Durvillaea antarctica (17.9 \pm 1.2 g/100 g DW), Caulerpa lentillifera (22.20 \pm 0.27 g/100 g DW), Gelidiella acerosa (10.3 \pm 4.9 g/100 g DW) and Sargassum wightii (25 \pm 2 g/ 100 g DW)^{23,34,35,42}.

Sulphate is a typical component of marine algal polysaccharides, which is related to specific aspects of ionic regulation as well as high salt concentration in the environment. These sulphated mucilages are typical of marine algae, and are not found in land plants. In brown algae, sulphate is derived from fucans, while in red algae it is derived from galactans. Seaweed dietary fibres are not digested by humans; particularly, sulphated algal polysaccharides are weakly fermented and poorly desulphated by colonic bacteria. In fact, seaweed dietary fibre polysaccharides generally retain their ionic groups throughout the digestive tract. This suggests that they are relatively innocuous regarding sulphate ion toxicity⁴¹. The sulphate content in this study varied slightly across the colour forms $(5.31\pm0.51 \text{ to } 5.89\pm0.00 \text{ g/100 g DW})$. A similar trend was observed by Hayashi et al.¹² who studied the four strains of the same species in the subtropical waters of São Paulo State, Brazil. The suphate content of the three colour forms studied in present study were higher than Fucus (3.75 g/100 g DW) and Fucus vesiculosus (2.4 g/100 g DW), as well (1.33 g/100 g DW), as, Laminaria Wakame

Table 2 — Mineral content of the three colour forms of *K. alvarezii*. Values are expressed as mean \pm standard deviation. ND = below the detection limit. The different superscript letters are significantly different at P < 0.05 [ANOVA with least significant difference (LSD)]

Mineral	Brown	Green	Pale yellow
Na	7.97 ± 0.29^a	6.63 ± 0.43^{b}	6.61 ± 0.25^{b}
Κ	9.40 ± 0.39^{a}	3.81 ± 0.48^b	$8.89\pm0.43^{\text{c}}$
Ca	$0.42\pm0.01^{\text{a}}$	$0.47\pm0.21^{\text{a}}$	$0.63\pm0.27^{\rm b}$
Mg	$1.01\pm0.02^{\rm a}$	$0.99\pm0.10^{\rm a}$	$1.17\pm0.12^{\rm b}$
Р	$12.11\pm0.08^{\rm a}$	4.72 ± 0.36^{b}	$1.57\pm0.07^{\rm c}$
Cd	$0.22\pm0.01^{\text{a}}$	$0.05\pm0.03^{\rm b}$	$0.31\pm0.01^{\text{c}}$
Pb	0.05 ± 0.01^{a}	$0.11\pm0.02^{\rm b}$	$0.10\pm0.08^{\rm b}$
As	0.37 ± 0.11^{a}	$0.22\pm0.08^{\rm b}$	$0.20\pm0.09^{\rm b}$
Hg	ND	ND	ND
Cr	$0.04\pm0.02^{\rm a}$	$0.05\pm0.01^{\rm a}$	$0.23\pm0.01^{\text{b}}$
Zn	3.31 ± 0.01^{a}	$1.68\pm0.02^{\rm b}$	$1.49\pm0.07^{\rm c}$
Cu	0.21 ± 0.01^{a}	$0.26\pm0.03^{\rm a}$	$0.92\pm0.03^{\rm b}$
Fe	2.57 ± 0.09^{a}	$0.52\pm0.06^{\rm b}$	$0.29\pm0.10^{\rm c}$
Mn	0.97 ± 0.01^{a}	$0.69\pm0.01^{\rm b}$	$1.70\pm0.05^{\rm c}$
Co	$0.06\pm0^{\rm a}$	$0.04\pm0^{\mathrm{a}}$	$0.16\pm0^{\mathrm{b}}$
Mo	$0.04\pm0.02^{\rm a}$	$0.03\pm0.06^{\rm a}$	$0.02\pm0.01^{\rm b}$
Ni	0.01 ± 0.02^{a}	$0.23\pm0^{\text{a}}$	0.16 ± 0.06^{b}

(1.43 g/100 g DW), *Chondrus* (5.86 g/100 g DW), and *Nori* (2.37 g/100 g DW)⁴¹.

Among the 17 minerals analyzed (Table 2), high K content was recorded in the brown form (9400 mg/ 100 g d wt) that had lowest Ni (0.01 mg/100 g d wt). However, the green form had high Na (6630 mg/100 g d wt) and lowest Mo (0.03 mg/100 g d wt). Similarly, the pale yellow form contained highest K (8890 mg/100 g d wt) and lowest Mo (0.02 mg/100 g d wt). The total mineral content of the brown, green and yellow form was 18820, 11909 and 17307 mg/100 g d wt, respectively. Figure 2 illustrates the comparison of macro-element concentrations of the three colour forms of K. alvarezii. There was a major difference in the phosphorus content amongst the three forms, wherein highest values were recorded in case of the brown colour form of K. alvarezii. In fact, the brown colour form was also rich in sodium and potassium. The sodium content of the green and the pale yellow colour forms were almost at par but the potassium content of the pale yellow form were much higher than the green colour form.

Indegaard & Minsaas⁴³ suggested the intake of algal products as dietary supplements and stated the daily intake of some trace elements for adults (Fe: 10 - 18 mg, Zn: 15 mg, Mn: 2.5 - 5.0 mg and Cu: 2 - 3 mg/100 g dry wt). Evaluation of the selected total micro-nutrient content (Fe+Zn+Mn+Cu) revealed maximum values in the brown colour form (7.06 ± 0.12 mg/100 g d wt), followed by pale yellow (4.4 ± 0.25 mg/ 100g d wt) and green (3.15 ± 0.12 mg/100 g d wt) forms. These values were nearly comparable with *Laminaria* (5.1 mg/100 g d wt)⁴¹.

Sodium content of *K. alvarezii* was higher than terrestrial vegetables like carrots, sweet corn, green peas, potato and tomato⁴⁴. As intake of sodium chloride and diets with high Na/K ratio are related to the incidence of hypertension, the Na/K ratio of three colour forms of *K. alvarezii* were determined. Here, the Na/K ratios ranging from 0.74 to 1.73 was obtained. Ortega-Calvo *et al.*⁴⁵ studied *Spirulina* and five other eukaryotic edible seaweeds used as food in Spain and reported Na/K ratios below 1.5 in all their seaweeds (0.33 – 1.34), which is intriguing from the point of nutrition; nevertheless, the Na/K ratio in olives and sausages are 43.63 and 4.89, respectively⁴⁶.

As, Cd, Cu, Pb, Cr, Ni and Zn are some other elements that were investigated keeping in mind the nutritional perspective; these in addition to Hg are of



Fig. 2 — Comparison of macro-element concentrations of the three colour forms of *K. alvarezii*. Values are expressed as mean \pm standard deviation. The different superscript letters on columns are significantly different at P < 0.05



Fig. 3 — Comparison of the toxic element concentration of the three colour forms of *K. alvarezii*. Values are expressed as mean \pm standard deviation. The different superscript letters on columns are significantly different at *P* < 0.05

immediate concern due to their potential toxicity for living organisms. Essentially, Hg was below detectable limit in the present study. Figure 3 illustrates the comparison of the toxic element concentration of the three colour forms of *K. alvarezii*. The pale yellow colour form showed highest Cd and Cr content amongst the three colour forms of *K. alvarezii*. Although highest As content was observed in case of the brown colour form, it had remarkably less Pb and Cr.

One can easily calculate the daily permissible dose for a food supplement depending on the presence of different toxic elements⁴⁷. In this study, based on the presence of various essential and toxic elements, a recommended daily intake of this edible seaweed is

Table 3 — Daily recommended intake of the three colour forms of <i>K. alvarezii</i> recommended based on the presence of toxic elements						
Elements	Permissible daily dose $(\mu g)^{(refs. 48,52)}$	Recommended daily intake of K. alvarerzii (g/day)				
	_	Brown	Green	Pale yellow		
Cd	50 - 150	22.73 - 68.18	100 - 300	16.13 - 48.39		
As	21	5.68	9.55	10.5		
Pb	250	500	227.3	250		
Cr	350	875	700	152.2		

provided in Table 3. Cu and Zn content was found to be in the range of 1.94 - 3.52 mg/100 g d wt in thesecolour forms which is below the toxic limit of consumption of macroalgae in human diet in countries like Japan and France⁴³. Based on toxicity of the above mentioned elements, it is recommended to use not more than 5.68 g d wt of brown form, 9.55 g d wt of green form and 10.5 g d wt of pale yellow form of K. alvarezii as a condiment. If 5.68 g d wt of brown form of K. alvarezii is consumed, the daily intake of investigated elements viz. Cd. As. Cu. Pb. Cr. Ni and Zn would be 12.5, 21.0, 11.93, 2.84, 2.27, 0.57 and 188 µg/day, respectively. However, the green and pale vellow forms of this seaweed differ slightly in the daily intake of these elements. When 9.55 g d wt of the green form is consumed, it would contain 4.78, 21.0, 24.83, 10.51, 4.78, 21.97 and 160.4 µg/day of Cd, As, Cu, Pb, Cr, Ni and Zn, respectively, while 10.5 g d wt of the pale yellow form contained 32.55, 21.0, 96.6, 10.5, 24.15, 16.8, and 156.5 µg of the same elements, respectively. These values are as per the tolerable daily intake of these elements established by the FAO/WHO Expert Committee⁴⁷⁻⁵⁰. Overall, the present study indicates the possibility of using these colour forms of K. alvarezii as a condiment in food supplements to improve the nutritive value of the human diet.

In a study important for strain selection, Dawes⁵¹ observed that the different colour types of *E. denticulatum* and *K. alvarezii* had different photosynthetic responses. Moreover, this study reveals the variation in biochemical constitution of the various colour forms of *K. alvarezii*. Therefore, it would be fascinating to carry out a study evaluating the eco-physiology of the various colour form and simultaneous evaluation of their biochemical constitution.

Conclusion

This study evaluating the proximate and mineral composition of three colour forms of *Kappaphycus alvarezii* (*viz.* brown, green and pale yellow), revealed significant differences in chemical constituents such as carbohydrates, proteins, lipid and mineral contents.

This is a one of its kind study on comparison of nutrient profiles of the three colour forms of *Kappaphycus alvarezii* in the Indian waters. The heavy metal content of the three colour forms were below the maximum permissible limit recommended by FAO/WHO, and they contained good amount of vital elements. Therefore, all three colour forms of *K. alvarezii* could serve as functional food with vital nutritional and biological values. Large scale use of this seaweed by the food industries could heighten the socio-economic status of farmers and fishermen.

Acknowledgements

Authors would like to thank the Director, Central Salt and Marine Chemicals Research Institute, Bhavnagar, for providing facilities, support and encouragement. The authors are also grateful to the Discipline Co-ordinator, Marine Algae and Marine Environment Discipline. Financial assistance by Department of Biotechnology, New Delhi, India (Sanction No: BT/PR 3309 / PID / 03 / 139 / 2002) is greatly acknowledged.

Conflict of Interest

The authors declare that they have no conflict of interests and all authors have given consent for publication.

Author Contributions

KSK: sampling, experimental procedures, data collection and analysis, editing the manuscript. SK prepared the draft of the manuscript. PVS: study concept, supervision, funding acquisition, reviewing the manuscript. All authors have read and agreed to the published version of the manuscript.

References

- Kumar C S, Ganesan P, Suresh P V & Bhaskar N, Seaweeds as a source of nutritionally beneficial compounds-a review, J Food Sci Technol, 45 (1) (2008) 1-13. https://oceanrep. geomar.de/id/eprint/29698
- 2 Paiva L, Lima E, Neto A I & Baptista J, Seasonal variability of the biochemical composition and antioxidant properties of *Fucus spiralis* at two Azorean Islands, *Mar Drugs*, 16 (8) (2018) 248. doi:10.3390/md16080248

- 3 Echave J, Lourenço-Lopes C, Carreira-Casais A, Chamorro F, Fraga-Corral M, et al., Nutritional composition of the Atlantic seaweeds Ulva rigida, Codium tomentosum, Palmaria palmata and Porphyra purpurea, Chem Proc, 5 (1) (2021) p. 07. https://doi.org/10.3390/CSAC2021-10681
- 4 Buschmann A H, Camus C, Infante J, Neori A, Israel Á H, et al., Seaweed production: overview of the global state of exploitation, farming and emerging research activity, Eur J Phycol, 52 (4) (2017) 391-406. https://doi.org/10.1080/ 09670262.2017.1365175
- 5 Suresh Kumar K, Ganesan K & Subba Rao P V, Seasonal variation in nutritional composition of *Kappaphycus alvarezii* (Doty) Doty an edible seaweed, *J Food Sci Technol*, 52 (5) (2015) 2751-2760. https://doi.org/10.1007/s13197-014-1372-0
- 6 Kumari P, Kumar M, Gupta V, Reddy C R K & Jha B, Tropical marine macroalgae as potential sources of nutritionally important PUFAs, *Food Chem*, 120 (2010) 749– 757. https://doi.org/10.1016/j.foodchem.2009.11.006
- 7 Kumar K S, Ganesan K & Rao P S, Seasonal variation in nutritional composition of *Kappaphycus alvarezii* (Doty) Doty—an edible seaweed, *J Food Sci Technol*, 52 (5) (2015) 2751-2760. https://doi.org/10.1007/s13197-014-1372-0
- 8 Guimaraes M, Estela M P & Destombe C, Green mutant frequency in natural populations of *Gracilaria domigensis* (Gracilariales, Rhodophyta) from Brazil, *Eur J Phycol*, 38 (2003) 155-159. https://doi.org/10.1080/0967026031-000085878
- 9 Van der Meer J P, Genetics, In: *Biology of the Red Algae*, edited by K M Cole & R G Sheath, (Cambridge University Press, Cambridge), 1990, pp. 103–119. doi:10.1046/j.1420-9101.1992.5030533.x
- Pereira D C, Trigueiro T G, Colepicolo P & Marinho-Soriano E, Seasonal changes in the pigment composition of natural population of *Gracilaria domingensis* (Gracilariales, Rhodophyta), *Revista Brasileira de Farmacognosia*, 22 (4) (2012) 874-880. http://dx.doi.org/10.1590/s0102695X 2012005000075
- 11 Hurtado-Ponce A Q, Carrageenan properties and proximate composition of three morphotypes of *Kappaphycus alvarezii* Doty (Gigartinales, Rhodophyta) grown at two depths, *Bot Mar*, 38 (1995) 215–219. https://doi.org/10.1515/ botm.1995.38.1-6.215
- 12 Hayashi L, de Paula E J & Chow F, Growth rate and carrageenan analyses in four strains of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) farmed in the subtropical waters of São Paulo State, Brazil, *J Appl Phycol*, 19 (2007) 393–399. https://doi.org/10.1007/s10811-006-9135-6
- 13 Wathelet B, Nutritional analysis for proteins and amino acids in beans (*Phaseolus* sp.), *Biotechnol Agron Soc Environ*, 3 (1999) 197–200. https://popups.uliege.be/1780-4507/index.php?id=15453
- 14 Dubois M, Gilles K A, Hamilton J K, Rebers P A & Smith F, Colourimetric methods for determination of sugars and related substances, *Anal Chem*, 28 (1956) 350–356. https://doi.org/10.1021/ac60111a017
- 15 Bligh E G & Dyer W J, A Rapid method of Total lipid Extraction and Purification, *Canadian J Biochem Physiol*, 37 (1959) 911–917. https://pubmed.ncbi.nlm.nih.gov/13671378/
- 16 AOAC, Official methods of analysis, 15th edn, (Association of Official Analytical Chemists, Arlington, VA, USA), 1990. http://www.fao.org/3/y4705e/y4705e24.htm

- 17 AOAC, Official Methods of Analysis, 16th edn, (Association of Official Analytical Chemists, Washington, DC, USA), 1995. https://searchworks.stanford.edu/view/4706329
- 18 Vogel A I & Bassett J, Vogel's text book of quantitative inorganic analysis-including Elementary instrumental analysis, 4th edn, edited by Bassett J, (Longman Group Limited, London), 1978, pp. 925.
- 19 Kumar K S, Ganesan K & Rao P S, Phycoremediation of heavy metals by the three-colour forms of *Kappaphycus alvarezii*, *J Haz Mat*, 143 (1-2) (2007) 590-592. https://doi.org/ 10.1016/j.jhazmat.2006.09.061
- 20 McDermid K J & Stuercke B, Nutritional composition of edible Hawaiian seaweeds, *J Appl Phycol*, 15 (2003) 513–524. https://doi.org/10.1023/B:JAPH.0000004345.31686.7f
- 21 Soriano E M, Fonseca P C, Carneiro M A A & Moreira W S C, Seasonal variation in the chemical composition of two tropical seaweeds, *Bioresour Technol*, 97 (2006) 2402–2406. https://doi.org/10.1016/j.biortech.2005.10.014
- 22 Wong K H & Cheung P C K, Nutritional evaluation of some subtropical red and green seaweeds, Part I. Proximate composition, amino acid profiles and some physico-chemical properties, *Food Chem*, 71 (2000) 475–482. https://doi.org/ 10.1016/S0308-8146(00)00175-8
- 23 Dawczynski C, Schubert R & Jahreis G, Amino acids, fatty acids, and dietary fibre in edible seaweed products, *Food Chem*, 103 (2007) 891–899. DOI: 10.1016/j.foodchem. 2006.09.041
- 24 Kumar M, Gupta V, Kumari P, Reddy C R K & Jha B, Assessment of nutrient composition and antioxidant potential of Caulerpaceae seaweeds, *J Food Compos Anal*, 24 (2011) 270–278. https://doi.org/10.1016/j.jfca.2010.07.007
- 25 El Din N G S & El-Sherif Z M, Nutritional value of some algae from the north-western Mediterranean coast of Egypt, J Appl Phycol, 24 (2012) 613–626. https://doi.org/10.1007/ s10811-012-9831-3
- 26 Mohammed H O, O'Grady M N, O'Sullivan M G, Hamill R M, Kilcawley K N, *et al.*, An Assessment of Selected Nutritional, Bioactive, Thermal and Technological Properties of Brown and Red Irish Seaweed Species, *Foods*, 10 (11) (2021) p. 2784. https://doi.org/10.3390/foods10112784
- 27 Rosenberg G & Ramus J, Ecological growth strategies in the seaweeds *Gracilaria follifera* (Rhodophyceae) and *Ulva* sp. (Chlorophyceae): soluble nitrogen and reserve carbohydrates, *Mar Biol*, 66 (1982) 251–259. https://doi.org/10.1007/ BF00397030
- 28 Sara Marsham S, Scott G W & Tobin M L, Comparison of nutritive chemistry of a range of temperate seaweeds, *Food Chem*, 100 (2005) 1331–1336. https://doi.org/10.1016/j. foodchem.2005.11.029
- 29 Kolb N, Vallorani L & Stocchi V, Chemical composition and evaluation of protein quality by amino acid score method of edible brown marine algae Arame (*Eisenia bicyclis*) and Hijiki (*Hijikia fusiforme*), Acta Aliment, 28 (1999) 213–222. https://core.ac.uk/download/pdf/78480971.pdf
- 30 Tee E S, Mohd Ismail N, Mohd Nasir I & Khatijah I, Nutrient composition of Malaysian foods. Kuala Lumpur: ASEAN Sub-Committee on Protein, *Food Habits Res Dev*, (1988) 250-258. https://www.yumpu.com/en/document/read/39334880/nutrient -composition-of-malaysian-foods-1988
- 31 Southgate D A T, Dietary fibre and health, In: *Dietary fibre: chemical and biological aspects*, edited by Southgate D A T,

Waldron K, Johnson I T & Fen-wick G R, (The Royal Society of Chemistry, Cambridge), 1990, pp. 10–19. https://doi.org/10.1177/146642409011000509

- 32 Mac Artain P, Christopher R G, Brooks M, Campbell R & Rowland I R, Nutritional value of edible seaweeds, *Nutr Rev*, 65 (2007) 535–543. https://doi.org/10.1111/j.1753-4887.2007.tb00278.x
- 33 Gupta S & Abu-Ghannam N, Bioactive potential and possible health effects of edible brown seaweeds, *Trends Food Sci Technol*, 22 (2011) 315–326. https://doi.org/10. 1016/j.tifs.2011.03.011
- 34 Robledo D & Freile-Pelegrin Y, Chemical and mineral composition of six potentially edible seaweed species of Yucatán, Bot Mar, 40 (1994) 301–306. https://doi. org/10.1515/botm.1997.40.1-6.301
- 35 Syad A N, Shunmugiah K P & Kasi P D, Seaweeds as nutritional supplements: analysis of nutritional profile, physicochemical properties and proximate composition of *G. acerosa* and *S. wightii, Biomed Prev Nutr*, 3 (2013) 139– 144. https://doi.org/10.1016/j.bionut.2012.12.002
- 36 Mushollaeni W, The physicochemical characteristics of sodium alginate from Indonesian brown seaweeds, *Afr J Food Sci*, 5 (6) (2011) 349–352. https://academicjournals.org /article/article1381313344_Mushollaeni.pdf
- 37 Fleury N & Lahaye M, Chemical and physio-chemical characterization of fibres from *Laminaria digitata* (Kombu Breton): a physiological approach, *J Sci Food Agr*, 55 (1991) 389–400. https://doi.org/10.1002/jsfa.2740550307
- 38 Ito K & Hori K, Seaweed: chemical composition and potential uses, *Food Rev Intl*, 5 (1989) 101–144. https://doi.org/10.1080/87559128909540845
- 39 Mabeau S & Fleurence J, Seaweed in food products: biochemical and nutritional aspects, *Trends Food Sci Technol*, 4 (1993) 103–107. https://doi.org/10.1016/0924-2244(93)90091-N
- 40 Abirami R G & Kowsalya S, Nutrient and nutraceutical potentials of seaweed biomass *Ulva lactuca* and *Kappaphycus alvarezii*, *J Agric Sci Technol*, 5 (1) (2011) 109-115.
- 41 Ahmad F, Sulaiman M R, Saimon W, Yee C F & Matanjun P, Proximate compositions and total phenolic contents of selected edible seaweed from Semporna, Sabah, Malaysia, *Borneo Sci*, 31 (2012) 74–83. https://jurcon.ums.edu.my/ojums/index.php/borneo-science/article/view/171

- 42 Rupérez P, Mineral content of edible marine seaweeds, *Food Chem*, 79 (2002) 23–26. https://doi.org/10.1016/S0308-8146(02)00171-1
- 43 Taboada C, Millán R & Míguez I, Composition, nutritional aspects and effect on serum parameters of marine algae Ulva rigida, J Sci Food Agric, 90 (2010) 445–449. https://doi.org/ 10.1002/jsfa.3836
- 44 Indegaard M & Minsaas J, Animal and human nutrition, In: Seaweed resources in Europe: uses and potential, edited by Guiry M D & Blunden G, (Chichester: John Wiley & Sons Ltd), 1991, pp. 21–64. https://doi.org/10.1002 /aqc.3270020206
- 45 Food and Nutrition Board, Food chemical codex, 3rd edn, (National Academy Press, Washington, DC), 1981. https://www.nap.edu/read/19642/chapter/3
- 46 Ortega-Calvo J J, Mazuelos C, Hermosin B & Saiz-Jimenez C, Chemical composition of *Spirulina* and Eukaryotic algae food products marketed in Spain, *J Appl Phycol*, 5 (1993) 425–435. https://doi.org/10.1007/BF02182735
- 47 USDA, Agricultural research service, Nutrient Database for Standard Reference, Release 14 (2002). https://www.ars.usda. gov/ARSUserFiles/80400535/DATA/sr14/sr14_doc.pdf
- 48 Concon J M, Food Toxicology, Part A, Vol. 582, (Marcel Dekker Inc, New York), 1988, pp. 1049–1073. https://doi.org/10.1002/food.19880321032
- 49 US EPA, IRIS: Integrated risk information system, (U.S. Environmental Protection Agency, Micromedex, Inc., Englewood, CO), 1996. https://www.epa.gov/iris
- 50 WHO & International Programme on Chemical Safety, Guidelines for drinking-water quality, Vol. 2, Health criteria and other supporting information, 2nd edn, (World Health Organization, Geneva), 1996. https://apps. Whoint.isis/ handle/10665/38551.
- 51 Dawes C J, Irradiance acclimation of the cultured Philippines seaweeds, *Kappaphycus alvarezii* and *Eucheuma denticulatum*, *Bot Mar*, 35 (1992) 189–195. https://doi.org/ 10.1515/botm.1992.35.3.189
- 52 Phaneuf D, Cote I, Dumas P, Ferron L A & Le Blanc A, Evaluation of the contamination of Marine Algae (Seaweed) from the St. Lawrence River and Likely to be consumed by Humans, *Environ Res Sec A*, 80 (1999) S175–S182. https://doi.org/10.1006/enrs.1998.3915