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ORIGINAL ARTICLE

Seasonal variation in nutritional composition of *Kappaphycus alvarezii* (Doty) Doty—an edible seaweed

K. Suresh Kumar · K. Ganesan · P. V. Subba Rao

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Abstract Seasonal variation in the proximate and mineral composition of *Kappaphycus alvarezii* were investigated in the present study, moreover, the relationship between the nutritive components of this seaweed and the environment were also established. Carbohydrates represented the major portion of the algae (i.e. average total carbohydrate content was 23.01±1.64 g/100 g DW), while the lipid content was the lowest among the constituents investigated (0.39±0.04 to 0.91±0.51 g/100 g DW). The protein content of *K. alvarezii* varied from 12.69±0.6 to 23.61±0.02 g/100 g DW, and the fiber content varied between 9.68±0.08 to 18.57±0.15 g/100 g DW. Highest total mineral content (29939.61±9340.38 mg/100 g DW) was observed in April 2005, while least values were recorded in January 2006 i.e. (10997.62±1120.26 mg/100 g DW). The Na/K ratio during the study ranged from 0.34 to 0.87. All the samples showed remarkable semi-refined carrageenan (SRC) yield ranging from 42.70±1.07 to 63.73±1.73 % (average 53.90±1.37 %), and, the samples collected during December 2004 and January 2006 demonstrated maximum gel strengths i.e. 743±15.28 and 783.33±15.28 g·cm^{-2} respectively. Various environmental parameters influenced the chemical composition of *K. alvarezii*, and these parameters demonstrated seasonal fluctuations. Moreover, based on the nutritional composition obtained, it could be stated that this seaweed has great scope to be incorporated into several food products as an excellent nutritional supplement, or as a value additive in animal or pet food.

Keywords Seaweed · *Kappaphycus alvarezii* · Proximate composition · Mineral composition · Seasonal variation

Introduction

Benthic marine macroalgae, commonly known as seaweeds, are one of the living renewable resources of the marine environment with potential food and therapeutic applications; they have been used directly or indirectly as human food in Asian countries and are considered under-exploited resources (Tseng 2004). Currently there is increasing consumer interest in products that can support or even promote health. Nutritionists acclaim seaweed as being low in calories, and rich in vitamins, minerals, and dietary fibers (Jimenez-Escrig and Sanchez-Muniz 2000).

Seaweeds have been conventionally used for a wide variety of applications such as food, fodder, fertilizer, for medicinal purpose, and phycocolloids. Approximately, 250 species of seaweeds have been commercially utilized worldwide, amongst which 150 species are favorably consumed as human food; however, in western countries they form a source of polysaccharides (agar, alginates, carrageenans) for food and pharmaceutical industry (Zemke-White and Ohno 1999; Kumari et al. 2010). In order to cope with the growing market demand of seaweeds, several of them are cultivated commercially as a source of livelihood worldwide.

Seaweeds generally show great variation in the nutrient contents, which could be related to several environmental factors such as water temperature, salinity, light and nutrients. Further, most of the environmental parameters influencing seaweed composition generally vary with season; moreover, the changes in ecological conditions can also stimulate or inhibit the biosynthesis of several nutrients (Soriano et al.

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2006). Seasonal variations in the chemical composition and nutritive value have been reported in common marine seaweed from Hong Kong (Kaehler and Kennish 1996) and Ireland (Mercer et al. 1993). Though there have been several reports on biochemical composition of various seaweeds across India, but there are very few studies focusing on the temporal variations in chemical composition of seaweeds in Indian context. This study aims at presenting the seasonal variation in the proximate and mineral composition of tropical Indian red seaweed *K. alvarezii*, a carrageenophyte with enormous commercial value.

Materials and methods

Seaweed: sampling and processing

Kappaphycus alvarezii was collected once in a month (from September 2004 to April 2006) from the cultivation site at Okha (22°28.656 N and 69°04.015 E) Gujarat, Northwest coast of India. The samples were thoroughly washed with seawater followed by fresh water, and subsequently dried at 60 °C in an oven, the dried samples were ground to particle size<1 mm and stored at room temperature in airtight plastic containers for further use.

Analysis of proximate composition

The nitrogen content of the dried seaweed was quantified using Kjeldahl procedure (Wathelet 1999) using KEL PLUS-KES 20L Digestion unit attached to a KEL PLUS-CLASSIC DX Distillation unit (M/s PELICAN Equipments, Chennai, India). The digestion was performed using sulfuric acid (96 % H_2SO_4; initially for 75 min at 420 °C, and again for 75 min at 370 °C); this was thereafter distilled with boric acid solution (2 %) and titrated with 0.1 M HCl. The crude protein content was estimated by multiplying the nitrogen with a factor of 6.25; while the total carbohydrate content was assayed by the phenol–sulphuric acid method using glucose as a standard (Dubois et al. 1956). Crude lipids were extracted using soxhlet extractor using 2:1 ratio of chloroform-methanol (Bligh and Dyer 1959). The fiber content of the dried seaweed was determined using a standard method outlined by AOAC (1990), here, the acid hydrolysis was carried out with sulfuric acid (0.3 N H_2SO_4) and the base hydrolysis was undertaken using sodium hydroxide (0.5 N NaOH). The cold extraction was performed with acetone. The sample was then dried (1 h at 110 °C) until it reached a constant weight, cooled in a desiccator, and weighed (W_1); thereafter, it was placed in a muffle furnace at 550 °C for 3 h, cooled (in a desiccator) and reweighed (W_2). The crude fiber percentage was calculated following the equation: % crude fiber = $(W_1 - W_2 /W_0) \times 100$ (wherein, W_0 was the initial weight of the dried seaweed which was 2 g). The ash content was analyzed by shade drying the samples at room temperature and later in an oven at 80 °C for 1 h, thereafter, one gram of the powdered sample was accurately taken in a crucible, ashed at 550 °C in muffle furnace for 6 h to a constant weight, and the ash obtained was then quantified gravimetrically (AOAC 1995).

Analysis of minerals by ICP-OES

One gram seaweed was ashed, moistened with ten drops of distilled water (Milli-Q) and carefully dissolved in 3 ml HNO_3 (1:1 v/v), followed by heating at 100–120 °C till the solution totally evaporated. The crucible was returned to muffle furnace and ashed again for 1 h at 550 °C and cooled. 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 μm) 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) (Suresh Kumar et al. 2007). Determination of mineral contents (Na, K, Ca, Mg, P, S, B, Cd, Co, Cr, Cu, Fe, Mn, Zn, Hg, Mo and V) of each seaweed sample was carried out using Inductively Coupled Plasma Optical Emission Spectroscopy, ICP-OES (Perkin-Elmer, Optima 2000). The analysis of all the above minerals was carried out in triplicate. Mean and standard deviation were calculated. All the chemicals and solvents used for experiments were of analytical grade.

Environmental parameters

Selected environmental parameters were determined at the farming site during the period of experimental cultivation (September 2004 to April 2006). All determinations, including water sample collections, were conducted at a depth of 30 cm i.e. the same depth as the cultured seaweeds. Seawater temperature was recorded daily throughout the study period and the mean monthly temperature was obtained. Salinity was determined fortnightly and the average value was recorded. The seawater samples were subjected to fortnightly nutrient analysis; here, dissolved inorganic phosphate ($P–PO_4$) and dissolved inorganic nitrate ($N–NO_3$) were determined according to the method described by Strickland and Parsons (1972) followed by which average monthly values were calculated.

Extraction of semi-refined carrageenan

The pre-cleaned dried seaweed (10 g) was rinsed with fresh water at ambient temperature and treated with 200 ml of 8 % KOH (cooking solution), in a container (glass) maintained at approximately 70 °C for 3 h. After 3 h of processing had elapsed, the seaweed was removed from the hot aqueous KOH solution and drained (this KOH mixture containing the seaweed was handled with extreme caution as it was corrosive having a pH ranging from 12 to 14). The seaweed was then subjected to a series of fresh water washes to reduce the pH, and, to wash residual KOH from the seaweed. This process helps eliminate certain salts and residual saponification products created during the KOH cook/reaction. The processed seaweed (containing carrageenan) obtained after the final rinse, was then chopped, dried and ground (Rideout and Bernabe 1997); this processed seaweed powder referred to as semi-refined carrageenan was then used for further determinations.

The carrageenan yield (%) was determined according to the formula:

$$\text{Yield\%} = (\text{Wc}/\text{Wm}) \times 100$$

Where, Wc is the extracted carrageenan weight (g) and Wm is the dry powder or algal weight (g) used for extraction. The data were then presented as the mean yield obtained from three replicates.

Gel strength measurement

In order to evaluate the gel strength of the product, 1 g of carrageenan was soaked in a solution of 1 % KCl for 2 h. This mixture was boiled (100 °C) for 20 min, cooled and left undisturbed at room temperature to facilitate the formation of gel; thereafter, the gels were refrigerated at 10 °C overnight (approx. 10 h) in a refrigerator. Gel strength was measured at 25 °C using a Nikkansui type gel tester (Kiya Seisakusho Ltd. Tokyo, Japan) as described by Hurtado-Ponce and Umezaki (1988) and it was expressed as g·cm^{-2}.

Statistical analysis

Analysis of variance (ANOVA) was conducted to determine the differences in seasonal variations of biochemical constituents; moreover multiple comparison tests with the least significance difference (LSD) were performed to determine significant differences.

Results and discussion

Seasonal fluctuations in various environmental parameters were observed during the study period. Apart from showing seasonal fluctuations in environmental parameters along the various coasts of India, several chemical oceanographic studies also reveal seasonal variations in nutrients and their interrelationships in the Northeastern Arabian Sea, and particularly the West coast of India (Sen Gupta et al. 1976). However, there are few studies highlighting the seasonal fluctuation in the various parameters influencing seaweed composition particularly the shoreline water of Okha coast. During the course of study a mean salinity and temperature were 34.20‰ and 26.55 °C respectively, which corresponded with reports of Gunalan et al. (2010) and Subba Rao et al. (2008). The seawater temperature varied from 22.5 to 30.1 °C, while the salinity fluctuated from 31.20 to 36.64‰. The tropical climate, warm, nutrient-enriched seawater, high light levels and high degree of water motion waters are suitable for farming *Kappaphycus* species, therefore, it could be projected that the prevalent environmental conditions encountered during this study were suitable for the growth of *Kappaphycus alvarezii*.

As evidenced in most studies, environmental parameters and season had a definite impact on the growth and biochemical constituent of *K. alvarezii*; Fig. 1 and 2 elucidate the seasonal variation in protein and carbohydrate content of *K. alvarezii* ($P<0.001$) respectively. Subba Rao et al. (2008) reported that the growth rate of *Kappaphycus alvarezii* significantly correlates with salinity, nitrate and seawater temperature at various sites; however, this study reveals that various environmental parameters had a vital impact on the chemical composition of this seaweed. The average protein content of this seaweed (19.25±0.15 g/100 g DW) was much higher than that reported for *Sargassum vulgare* (4.59–9.97 g/100 g DW; Soriano et al. 2006), *Ulva lactuca* (7.06±0.06 g/100 g DW; Wong and Cheung 2000), *Undaria pinnatifida* (7.5±1.9 g/100 g DW; Dawczynski et al. 2007), *Caulerpa veravelensis* (7.77±0.59 g/100 g DW; Kumar et al. 2011), *C. lentillifera* (9.26±0.03 g/100 g DW; Nguyen et al. 2011), *Caulerpa scalpelliformis* (10.50±0.91 g/100 g DW; Kumar et al. 2011), *Laminaria* sp. (11.6±0.8 g/100 g DW; Dawczynski et al. 2007), *C. racemosa* (12.88±1.17 g/100 g DW; Kumar et al. 2011), *Hizikia fusiforme* (13.8±6.2 g/100 g DW; Dawczynski et al. 2007) and *Ulva rigida* (17.8 g/100 g DW; Taboada et al. 2010). However, these values were comparable with that of *Hypnea charoides* (18.4±0.30 g/100 g DW; Wong and Cheung 2000), *Hypnea japonica* (19.0±0.3 g/100 g DW; Wong and Cheung 2000), and *Gracilaria cervicornis* (14.3–22.71 g/100 g DW; Soriano et al. 2006). El Din and El-Sherif (2012) reported the protein content of *C. prolifera*, *C. racemosa*, *C. bursa*, *H. tuna*, *U. petiolata*, *Udotea* sp., *G. verrucosa*, *R. ardissonei*, *C. spinosa*, *D. dichotoma*, *S. acinarium*, *S. hornschuchii* and *S. vulgare* to be 3.86, 8.23, 5.35, 11.8, 11.5, 14.5, 7.08, 4.5, 7.01, 8.15, 3.9, 6.05 and 6.15 g/100 g DW respectively, however, these values are lower than that obtained for *K. alvarezii* in the present study. Moreover, *K. alvarezii* contained relatively higher protein as compared to the seaweeds species habitually consumed as food (Fleurence 1999). Dawes (1998) vividly state that the members of Rhodophyta are characterized by greater protein content when compared to those of Phaeophyta, this could probably explain the high protein content in the species studied herein. Additionally, the mean protein content of *K. alvarezii* (19.25±0.15 g/100 g DW) was higher

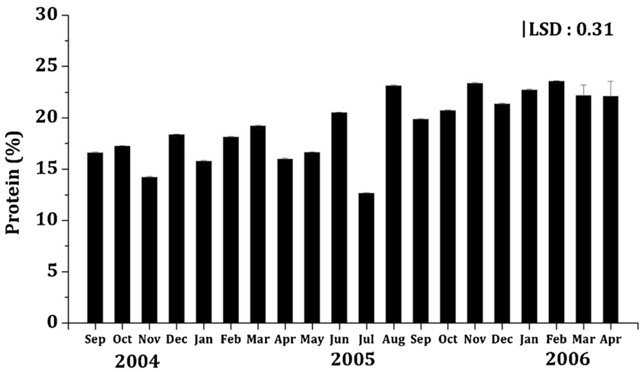

Fig. 1 Seasonal variation in protein content (%) of *K.alvarezii*

than that reported for higher plants (Norziah and Ching 2000). However, reports suggest that although chemical composition of seaweeds could be influenced by factors such as species, habitat, maturity, geographical locations, environmental parameters, and season (Ito and Hori 1989; Fleurence 1999), it is essential to note the very presence of proteins and other vital nutrients such as high concentrations of essential amino acids (EAA) (e.g. argenine, aspartine and glutamine found in many seaweed species; Fleurence 1999) make them a promising candidate to be used in various food formulations or as a supplement. More essentially, even though there may be seasonal variations in the protein content and in turn the essential amino acids, however, these EAA are available throughout the year despite seasonal variations in their concentrations (Galland-Irmouli et al. 1999).

The average protein content of *K. alvarezii* (19.25±0.15 g/100 g DW), was much less than the mean carbohydrate content (25.87±1.64 g/100 g DW). The carbohydrate content of *K. alvarezii* (average 25.87±1.64 g/100 g DW) was higher than several seaweeds, for e.g. *Ulva rigida*, *Ulva lactuca*, *Caulerpa racemosa*, *Sargassum filipendula* and *Hypnea japonica* (Rosenberg and Ramus 1982), *C. prolifera*, *C. racemosa*, *C. bursa*, *H. tuna*, *U. petiolata*, *Udotea* sp., *G. corneum*, *G. verrucosa*, *R. ardissonei*, *C. spinosa*, *D. dichotoma*, *S. acinarium*, *S. hornschuchii* and *S. vulgare* (El Din and El-Sherif 2012) which are reported to contain 6.40, 7.06, 3.98, 3.73, 4.28, 9.00, 6.93, 7.43, 7.40, 8.60, 8.00, 12.03, 15.77, 5.00, 20.91, 8.60, 13.67, 16.50 and 14.30 g/100 g DW of carbohydrate respectively. It is essential to note that, in the present study the occurrence of maximum carbohydrate content of *K. alvarezii* coincided with occurrence of maximum biomass, thereby suggesting a link between seaweed growth and carbohydrate content; similarly, Rosenberg and Ramus (1982) also related the carbohydrate synthesis to periods of maximum growth, increased photosynthetic activity and a reduction in protein content. Moreover, during the period of occurrence of maximum carbohydrate in *K. alvarezii* higher values of water temperature, salinity and sunlight intensity were recorded, which confirms the influence of these parameters on carbohydrate synthesis (Munda and Kremer 1977). However, a negative correlation was found in this study between total carbohydrate and phosphate (r=−0.471). On the other hand, an inverse relationship was observed between carbohydrate and protein content; Givernaud et al. (1993) observed a similar a pattern for several species of seaweeds.

The lipid content varied from 0.39±0.04 to 0.91±0.51 g/100 g DW (Fig. 3) with an average of 0.64±0.08 g/100 g DW. The lipid content of marine macroalgae (seaweed) has been reported to generally range between 1 and 6 g/100 g DW (Dawczynski et al. 2007). However, in the present investigation on the carragenophytic seaweed *K. alvarezii*, the lipid content ranged from 0.39±0.04 to 0.91±0.51 g/100 g DW (Fig. 3) which was comparable with *Sargassum polyschides* (0.7±0.09 g/100 g DW), *Corallina officinalis* (0.3±0.2 g/100 g DW; Sara Marsham et al. 2005) as well as *Hizikia* sp. and Arame (0.7–0.9 g/100 g DW; Kolb et al. 1999); however, it was lower than *Palmaria* sp. (1.8±0.14 g/100 g DW), *Hypnea elongata* (0.97±0.07 g/100 g DW) and *Undaria pinnatifida* (1.05±0.01 g/100 g DW) (Sanchez-Machado et al. 2004). Moreover, there was a positive correlation between the water temperature and the lipid content (r=0.554).

Seaweeds are reported to be rich in fiber as compared to most fruits and vegetables; moreover, consumption of seaweeds helps increase the intake of dietary fiber and lower the occurrence of some chronic diseases (diabetes, obesity, heart diseases, cancers, etc.). In addition, consumption of seaweed is known to promote the growth of beneficial intestinal flora and also protect them; it also reduces the overall glycemic response, greatly increases stool volume and reduces the risk of colon cancer (Southgate 1990; Gupta and Abu-Ghannam 2011). *K. alvarezii* was particularly rich in fiber. Its fiber content ranged from 9.68±0.08 to 18.57±0.15 g/100 g DW (Fig. 4). This was higher than that reported by Robledo and Freile-Pelegrin (1994); they reported *Sargassum vulgare*

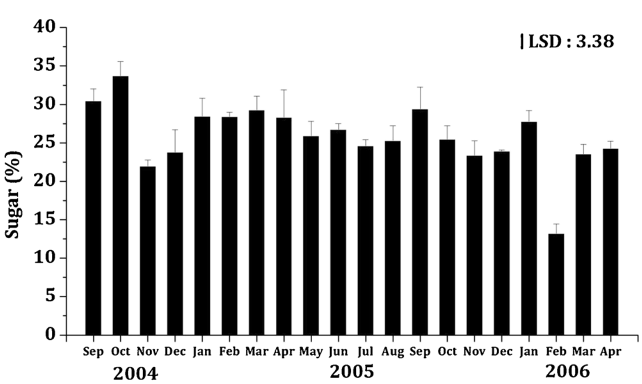

Fig. 2 Seasonal variation in sugar content (%) of *K.alvarezii*

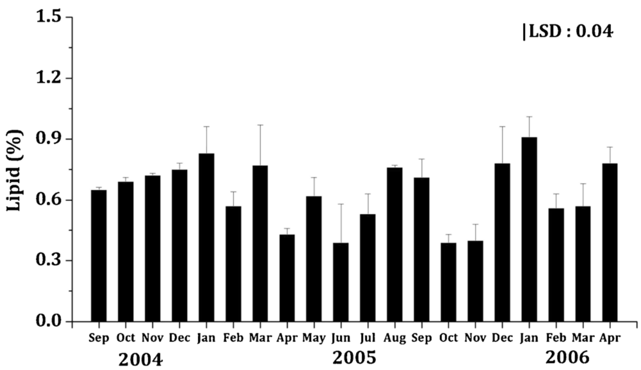

Fig. 3 Seasonal variation in lipid content (%) of *K.alvarezii*

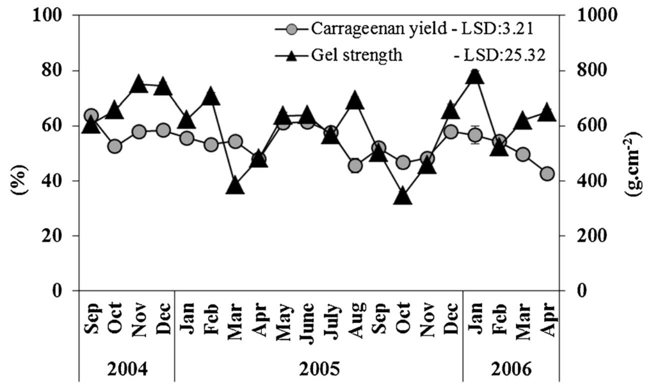

Fig. 5 Seasonal variation in semi-refined carrageenan yield (%) and gel strength (g·cm^{-2})

(mean 7.40±1.61 g/100 g DW) and *Gracilaria cervicornis* (mean 5.65±0.74 g/100 g DW) to have fiber content comparable to few other brown and red macroalgal species. On the other hand, the average fiber content of *K. alvarezii* (14.52±0.11 g/100 g DW) was comparable with *Gelidiella acerosa* (13.45±1.07 g/100 g DW) and lower than *Sargassum wightii* (17±1.19 g/100 g DW) (Syad et al. 2013).

The average SRC yield obtained in the present study (53.90±1.37 %) was comparable with western Pacific commercial sample (57 %; Hayashi et al. 2007). Subba Rao et al. (2008), cite several reports mentioning lower semi-refined carrageenan content (31–43 %) for the same seaweed farmed in the subtropical waters of Sao Paulo State, Brazil, and moderate yields for materials from Vietnam (34.5 to 45.3 %; Ohno et al. 1996) and Indonesia (45 %; Ohno et al. 1996), however, higher yields are reported from Philippines (54.6 %; Trono and Ohno 1986). On the other hand, the SRC obtained in this study also demonstrated remarkable gel strength ranging from 348.33±12.58 to 783.33±15.28 g·cm^{-2} with an average of 602.00±12.13 g·cm^{-2} which justifies its potential use in several pet food products. All the *K. alvarezii* samples yielded good amount of SRC i.e. the SRC yields ranged between 42.70±1.07 to 63.73±1.73 % (Fig. 5). A positive correlation could be derived between the carrageenan yield and gel strength (r=0.578; Table 1); nonetheless, the nitrate present in seawater had a positive correlation with carrageenan yield (r=0.465).

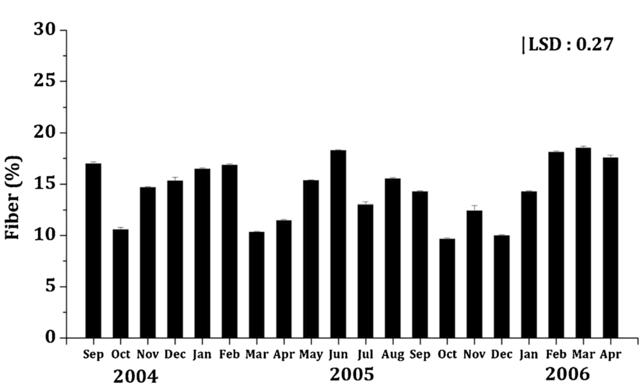

Fig. 4 Seasonal variation in fiber content (%) of *K.alvarezii*

The ash content of *K. alvarezii* ranged from 20.99±0.08 to 33.81±1.71 g/100 g DW (Fig. 6). The ash contents of the seaweeds are usually much higher than land vegetables other than spinach (Rupeŕez 2002; Omotoso 2006). However, the ash content is known to vary with species, geographical locations and season (Kaehler and Kennish 1996). The average ash content of *K. alvarezii* recorded in this study (27.00±1.62 g/100 g DW; Fig. 6) 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±0.1 g/100 g DW), *Durvillaea Antarctica* (17.9±1.2 g/100 g DW), *Caulerpa lentillifera* (22.20±0.27 g/100 g DW), *Gelidiella acerosa* (10.3±4.9 g/100 g DW) and *Sargassum wightii* (25±2 g/100 g DW) (Dawczynski et al. 2007; Mushollaeni 2011; Taboada et al. 2010; Syad et al. 2013). Generally, the ash content of a sample is known to reflect its mineral content. Mushollaeni (2011) state that the presence of ash content in seaweed showed that there were mineral salt in the sample; similarly, this study also confirms that *K. alvarezii* (which demonstrated high ash content) was also rich in minerals.

Seaweed are rich (8–40 %) in several essential minerals and trace elements required for human nutrition (Indegaard and Ostgaard 1991); in fact, several reports suggest that the mineral content in seaweeds is higher than edible land plants (Ortega-Calvo et al. 1993; USDA 2001). However, it is obvious that occurrence and amounts of these may vary with species, moreover, within the same species there may also be seasonal fluctuations in the composition of these elements. Seasonal variation in mineral composition of *K. alvarezii* have been elucidated in Table 2; the highest mineral content (29939.61±9340.38 mg/100 g DW) was recorded in the month of April 2005, while the lowest value (10997.62±1120.26 mg/100 g DW) was recorded in January 2006. Among the 17 minerals analyzed, *K. alvarezii* demonstrated highest S content (11,240±730 mg/100 g DW), and lowest

Table 1 Correlation coefficients (n=20) of the environmental parameters and the chemical composition of *K. alvarezii*

	Salinity	Phosphate	Nitrate	Biomass	Gel strength	Carrageenan Yield	Protein	Carbohydrate	Lipid	Fiber	Ash
Water °C	0.314	−0.169	0.139	0.005**	0.338	0.175	0.152	−0.042	0.554*	0.170	−0.259
Salinity		0.197	−0.216	0.193	−0.136	−0.426	0.373	−0.352	0.041	−0.127	0.077
Phosphate			0.380	−0.346	−0.278	−0.008	−0.174	−0.471*	−0.267	−0.076	0.071
Nitrate				−0.307	0.399	0.465*	−0.698**	0.004	0.055	0.017	−0.049
Biomass					−0.059	−0.196	0.140	−0.171	0.113	0.367	0.234
Gel strength						0.578*	−0.246	0.095	0.423	0.191	−0.155
Carrageenan yield							−0.476*	0.097	0.167	0.093	0.116
Protein								−0.355	0.023	0.144	0.046
Sugar									0.173	−0.313	−0.153
Lipid										0.052	0.196
Fiber											−0.205
Ash											1.000

*Significance (p<0.05)

**Significance (p<0.01)

quantities of Mo (0.04±0.02 mg/100 g DW). Apart from remarkable macro-mineral content (Na + K + Ca + Mg; 7,910±2,950 mg/100 g DW), *K. alvarezii* was also rich in selected micronutrients (Fe + Zn + Mn + Cu; 69.61±0.16 mg/100 g DW). The accumulation of Na and K salts generally depends upon the physiology as well as season; in this study the Na/K ratio of *K. alvarezii* ranged from 0.34 to 0.87. The macro-mineral content (Na + K + Ca + Mg) of *K. alvarezii* (7,910±2,950 mg/100 g DW) was lower than the values reported for the brown seaweeds such as *Fucus* (11,723±126 mg/100 g DW), *Laminaria* (17,061±182 mg/100 g DW), Wakame (17,875±382 mg/100 g DW), and for the red seaweeds such as *Chondrus* (8,606±90 mg/100 g DW) and Nori (8,082±214 mg/100 g DW) (Indegaard and Ostgaard 1991); however, it was higher than many land vegetables (carrot 3,276 mg/100 g DW, sweet corn 1,347 mg/100 g DW, potato 6,015 mg/100 g DW, tomato 3,429 mg/100 g DW), but spinach (9679 mg/100 g DW) is exceptional. Looking into the Mg content of all the samples, it could be

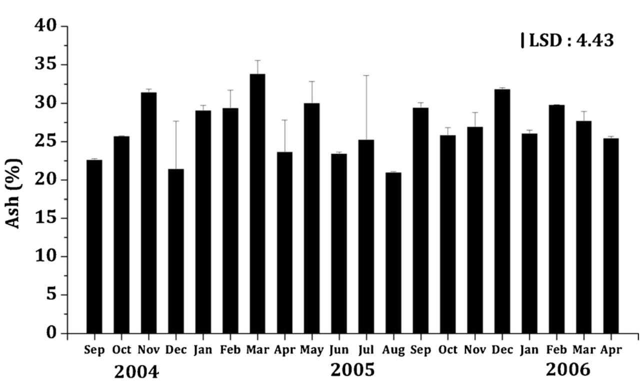

Fig. 6 Seasonal variation in ash content (%) of *K.alvarezii*

stated that *K. alvarezii* could prove to be a good source for Mg, which is essential for regulating central nervous system excitability and normal functions. In this context, Syad et al. (2013) reported that low levels of Mg contribute to the heavy metal deposition in the brain that leads Parkinson's, multiple sclerosis and Alzheimer's disease; hence inclusion of this seaweed in the diet could evade these mentioned problems. Marine algae are also interesting candidates as Fe sources, especially in countries where the algal production is feasible; *K. alvarezii* also comprised significant quantities of Fe. In this study, the selected micronutrients (Fe + Zn + Mn + Cu) in the seaweed was higher (69.61±0.16 mg/100 g DW) than the land plant sweet corn (Fe + Zn + Mn + Cu, 4.9 mg/100 g DW) and the seaweed *Laminaria* (5.1 mg/100 g DW) (Food and Nutrition Board 1981).

K. alvarezii showed a relatively higher sodium content than as compared to that reported for terrestrial vegetables (carrots, sweet corn, green peas, potato and tomato) (Food and Nutrition Board 1981). Generally, the intake of sodium chloride and diets with high Na/K ratio are related to the incidence of hypertension; here, the Na/K ratio of *K. alvarezii* ranged from 0.34 to 0.87. Ortega-Calvo et al. (1993) studied *Spirulina* and five other eukaryotic seaweeds used in food in Spain, and reported Na/K ratios were below 1.5 in all seaweeds studied (0.33–1.34), which is interesting from the point of nutrition; nevertheless, the Na/K ratio in olives and sausages are 43.63 and 4.89, respectively (USDA 2001).

Seaweeds have been investigated for various elements and heavy metals in the past; in fact, reports suggest that most of the trace elements present in the algal biomass are heavy metals (As, Cd, Cu, Hg, Pb and Zn), but their content is generally below the toxic limit in most seaweeds (Ortega-Calvo et al. 1993). However, it is essential to quantify the

Table 2 Macro (g/100 g DW), micro and trace elements (mg/100 g DW) of *K.alvarezii* determined by ICP-OES

Mineral	Sep-04	Oct-04	Nov-04	Dec-04	Jan-05	Feb-05	Mar-05	Apr-05	May-05	June-05	July-05	Aug-05
Na	2.49±0.20	1.11±0.29	2.56±0.17	3.79±0.17	3.27±0.25	2.38±0.36	2.61±0.27	2.71±0.31	2.05±0.1	2.07±0.17	2.06±0.25	2.56±1.53
K	3.40±0.17	2.93±0.21	4.08±0.23	6.41±0.17	5.27±0.30	4.96±0.46	5.85±0.31	6.69±0.26	3.46±2.08	5.21±0.2	3.5±0.12	7.59±1.53
Ca	0.82±0.33	0.82±0.17	0.80±0.06	0.96±0.12	0.80±0.23	0.81±0.15	0.83±0.23	0.76±0.42	0.98±0.25	0.89±0.06	0.89±0.16	0.75±2.52
Mg	0.74±0.18	0.66±0.24	0.69±0.17	0.84±0.12	0.91±1.16	0.77±0.71	0.71±0.12	0.78±0.15	0.92±0.21	0.76±0.12	0.89±0.46	0.72±0.3
P	0.10±0.00	0.09±0.00	0.11±0.00	0.13±0.00	0.17±0.00	0.17±0.00	0.12±0.00	0.10±0.00	0.13±0.00	0.12±0.00	0.17±0.00	0.15±0.00
S	5.43±0.46	11.38±0.20	11.37±0.35	13.76±0.52	13.06±0.50	13.47±0.79	15.39±0.64	9.59±0.46	12.13±0.46	11.84±0.46	12.18±0.17	18.03±3.46
Total	12.98±1.13	16.96±1.11	19.61±0.98	25.89±1.1	23.48±2.44	22.56±2.47	25.51±1.57	20.63±1.6	19.67±3.1	20.89±1.01	19.65±1.16	29.8±9.34
B	5.75±0.02	1.74±0.03	0.19±0.01	0.60±0.02	2.06±0.02	5.68±0.04	5.81±0.02	6.14±0.02	2.03±0.02	4.56±0.02	5.65±0.02	4.67±0.02
Cd	1.35±0.01	1.54±0.01	0.74±0.02	0.80±0.01	3.96±0.03	2.07±0.03	0.63±0.02	0.83±0.02	1.53±0.01	1.85±0.02	0.92±0.02	0.58±0.02
Co	0.48±0.02	0.48±0.01	0.71±0.01	0.64±0.02	0.20±0.01	0.76±0.03	0.19±0.02	0.18±0.02	2.65±0.03	0.16±0.03	2.14±0.02	0.28±0.02
Cr	2.66±0.02	4.31±0.02	5.36±0.01	7.28±0.01	0.74±0.03	3.59±0.02	1.73±0.15	1.35±0.04	13.47±0.31	1.53±0.03	13.54±0.01	5.34±0.04
Cu	0.62±0.03	1.03±0.02	1.07±0.02	0.76±0.01	0.76±0.07	0.65±0.03	0.58±0.02	0.55±0.06	3.77±0.01	0.38±0.01	0.65±0.02	0.58±0.02
Fe	49.16±0.09	68.80±0.06	94.50±0.17	98.85±0.01	132.73±0.04	101.64±0.08	34.57±0.03	24.67±0.02	111.83±0.12	27.47±0.25	64.78±0.01	123.6±0.17
Mn	1.35±0.02	0.16±0.02	2.26±0.04	1.67±0.01	0.66±0.04	1.67±0.02	1.03±0.02	0.75±0.03	1.24±0.01	0.79±0.01	0.69±0.01	1.6±0.02
Zn	2.21±0.02	1.07±0.02	2.26±0.01	1.34±0.03	1.65±0.04	2.25±0.02	2.17±0.21	1.45±0.02	2.26±0.05	1.4±0.02	1.43±0.02	2.76±0.03
Hg	0.02±0.01	0.04±0.01	0.22±0.01	0.09±0.01	0.04±0.01	0.02±0.01	0.02±0.02	0.01±0.01	0.08±0.01	0.05±0.01	0.07±0.0	0.05±0.0
Mo	0.05±0.01	0.16±0.01	0.01±0.01	0.10±0.01	0.05±0.03	0.06±0.05	0.03±0.01	0.02±0.12	0.03±0.01	0.03±0.01	0.02±0.01	0.01±0.01
V	0.11±0.01	1.78±0.02	0.33±0.02	0.37±0.12	0.04±0.04	0.11±0.09	0.11±0.01	0.08±0.01	0.1±0.1	0.08±0.01	0.08±0.01	0.14±0.03
Total	63.75±0.24	81.11±0.23	107.65±0.32	112.49±0.26	142.9±0.35	118.51±0.42	46.87±0.50	36.03±0.34	138.99±0.68	38.3±0.42	89.97±0.15	139.61±0.38
Grand Total (mg/ 100 g)	13043.75± 1130.24	17041.11± 1110.23	19717.65± 980.32	26002.49± 1100.26	23622.90± 2440.35	22678.51± 2470.42	25556.87± 1570.50	20666.03± 1600.34	19808.99± 3100.68	20928.30± 1010.42	19739.97± 1160.15	29939.61± 9340.38

Mineral	Sep-05	Oct-05	Nov-05	Dec-05	Jan-06	Feb-06	Mar-06	Apr-06	Average	LSD
Na	2.13±0.15	2.71±0.15	2.34±0.93	1.92±0.61	1.39±0.15	1.62±0.15	1.13±0.58	1.65±0.38	2.23±0.36	1.10
K	3.26±0.38	3.28±0.31	2.68±0.25	2.78±0.64	2.06±0.1	3.01±0.06	3.01±0.15	2.42±0.82	4.10±0.44	2.60
Ca	0.97±0.36	1.12±0.35	0.92±0.2	0.99±0.15	0.76±0.21	0.55±0.17	0.53±0.21	0.88±0.18	0.84±0.33	0.25
Mg	0.76±0.16	0.77±0.2	0.75±0.21	0.76±0.3	0.56±0.2	0.65±0.58	0.54±0.15	0.66±0.62	0.74±1.82	0.25
P	0.12±0.00	0.12±0.00	0.25±0.00	0.05±0.00	0.09±0.00	0.06±0.00	0.10±0.00	0.11±0.00	0.12±0.00	0.07
S	13.67±2.04	11.3±0.79	9.24±0.35	10.36±0.76	6.12±0.46	9.23±0.3	8.96±0.79	8.24±0.61	11.24±0.73	5.23
Total	20.91±3.09	19.3±1.8	16.18±1.94	16.86±2.46	10.98±1.12	15.12±1.26	14.27±1.88	13.96±2.61	19.27±3.68	
B	4.87±0.02	2.16±0.04	0.16±0.03	0.93±0.04	0.33±0.06	1.07±0.12	1.23±0.06	0.63±0.05	2.81±0.03	3.70
Cd	1.03±0.12	0.67±0.03	0.4±0.01	0.22±0.02	0.23±0.06	0.24±0.01	0.23±0.02	0.23±0.02	1.00±0.03	1.47
Co	0.6±0.02	0.47±0.06	0.76±0.02	0.13±0.06	0.1±0.03	0.14±0.01	0.06±0.01	0.12±0.03	0.56±0.02	1.11
Cr	5.26±0.02	1.57±0.02	5.16±0.03	0.87±0.03	1.14±0.0	1.33±0.23	0.37±0.01	1±0.02	3.88±0.05	6.3
Cu	0.55±0.03	0.55±0.03	0.77±0.02	0.6±0.1	0.35±0.02	0.22±0.01	0.19±0.02	0.47±0.02	0.76±0.03	1.22
Fe	90.2±0.02	45.5±0.03	126.77±0.23	48.56±0.02	13.83±0.03	21.15±0.02	9.09±0.02	31.19±0.02	65.94±0.07	81.78
Mn	1.28±0.04	1.17±0.04	1.48±0.02	1.25±0.02	0.5±0.01	0.57±0.01	0.36±0.02	0.88±0.02	1.10±0.02	0.88

Table 2 (continued)

Mineral	Sep-05	Oct-05	Nov-05	Dec-05	Jan-06	Feb-06	Mar-06	Apr-06	Average	LSD
Zn	1.8±0.1	1.77±0.02	3.37±0.02	2.96±0.04	1.04±0.01	1.09±0.01	0.81±0.02	1.99±0.03	1.85±0.04	1.16
Hg	0.01±0.0	0.01±0.01	0.1±0.01	0.02±0.01	0.02±0.03	0.01±0.01	0.02±0.01	0.02±0.01	0.05±0.01	0.08
Mo	0.04±0.02	0.07±0.01	0.01±0.01	0.05±0.01	0.03±0.0	0.03±0.01	0.02±0.01	0.04±0.01	0.04±0.02	0.06
V	0.16±0.02	0.13±0.01	0.15±0.02	0.13±0.01	0.05±0.01	0.07±0.01	0.05±0.02	0.09±0.01	0.21±0.03	0.62
Total	105.8±0.41	54.07±0.3	139.13±0.42	55.72±0.36	17.62±0.26	25.92±0.45	12.43±0.22	36.66±0.24	78.20±0.35	
Grand Total (mg/100 g)	21015.80±3090.04	19354.07±1800.30	16319.13±1940.42	16915.72±2460.36	10997.62±1120.26	15145.92±1260.45	14282.43±1880.22	13996.66±2610.24		

Table 3 Recommended dietary allowances of macronutrients, minerals and trace elements of *K. alvarezii*

Macronutrients, minerals and trace elements	Recommended Dietary Allowances per day[a]	Average content per gram in *K. alvarezii*
Macronutrients (g)		
Protein	9–71	0.17
Carbohydrate	60–210	0.23
Fiber	19–29	0.12
Fats	ND	0.007
Macro elements (mg)		
Boron	<20	0.03
Calcium	210–1,000	8.42
Iron	6–27	0.63
Magnesium	30–320	7.42
Manganese	0.003–2.6	0.011
Phosphorus	100–1,250	1.23
Zinc	2–12	0.019
Microelements (μg)		
Cadmium	50–150	10.02
Chromium	5.5–45	38.87
Copper	200–1,300	7.55
Molybdenum	2–50	43
Mercury	40	0.46

[a] According to Dietary Reference Intakes (2004), Food and Nutrition Board (1989), Recommended Daily Intakes of Various Food Supplements (2007), Concon (1988) and Phaneuf et al. (1999)

mineral, ash and heavy metal content in any seaweed before recommending its use in food formulation. In the USA, algal products have to comply with the following maximum limits: 45 % ash, 40 ppm heavy metals (Dietary Reference Intakes 2004). In the present study the Cu + Zn content ranged from 1.47 to 4.31 mg/100 g DW, which is below the toxic limits permitted for macroalgae for human consumption (Ortega-Calvo et al. 1993). Indegaard and Ostgaard (1991) suggested incorporation of algal products in the daily diet of adults, and recommend intake values of some trace elements (Fe: 10–18 mg, Zn: 15 mg, Mn: 2.5–5 mg and Cu: 2–3 mg. 100 g dry weight). Table 3 provides a comparison of the various recommended dietary allowances of macronutrients, minerals and trace elements and their presence in *K. alvarezii* (1 g); as elucidated this seaweed indeed qualifies to be incorporated as a food ingredient.

Based on the study conducted herein, it could be stated that *K. alvarezii* could be an excellent source of essential macronutrients and minerals, therefore, it could probably compensate for the frequently low minerals content of food; moreover, it also comprises significant quantities of protein and fiber. Hence, it could be preferentially used as an essential value-added food supplement or spice to the vegetarian or omnivorous diet.

Conclusion

Seaweeds have been traditionally used for variegated purposes including several food applications. Studies on temporal variations in the proximate and mineral composition of *K. alvarezii* conducted herein reveal the consistent presence of fiber and minerals (micro and macro nutrient) in this widely cultivated seaweed, thereby suggesting its use as a potential food supplement. *K. alvarezii* having high nutritional value, could either be used in the food industry as a source of these essential ingredients, or could be used as such for oral consumption after proper processing. However, its commercial value could also be enhanced by improving its quality and further increasing the probability of its use in a wider range of novel seaweed-based products. Nevertheless, it could definitely be incorporated into various animal and pet food products as a vital value additive supplement.

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