



# Seasonal variation of nutritional and antioxidant properties of different *Kappaphycus alvarezii* strains (Rhodophyta) farmed in Brazil

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## Abstract

Antioxidant potential, carbohydrate content, ash, minerals, proteins, and amino acids of *Kappaphycus alvarezii* farmed along the São Paulo coast, Brazil, were evaluated to support the best use of four strains and new applications with added value. Ash content ranged from 25.60 to 11.65%. Mineral contents varied from  $10,130.90 \pm 1,613.78$  mg (100 g)<sup>-1</sup> DW (summer 2018) to  $12,561.20 \pm 2,190.72$  mg (100 g)<sup>-1</sup> DW (summer 2017), and the highest mineral contents occurred in the green strain. Carbohydrate levels varied from  $122.92 \pm 15.11$  mg g<sup>-1</sup> DW (summer 2017) to  $231.79 \pm 16.86$  mg g<sup>-1</sup> DW (winter 2017), and the highest carbohydrate value was observed in the G11 strain. The highest protein amount was observed in the brown strain with 8.79 mg (100 g)<sup>-1</sup> DW. The highest antioxidant potential of *K. alvarezii* was in spring 2017 for the brown strain. Total phenolic content ranged from  $41.77 \pm 15.41$  to  $366.58 \pm 109.17$  mg GAE g<sup>-1</sup> DW, DPPH activity ranged from  $13.29 \pm 1.20$  to  $61.07 \pm 3.43\%$ , FRAP ranged from  $58.73 \pm 3.96$  to  $105.54 \pm 6.60\%$ , and ABTS varied from  $95.29 \pm 4.31$  to  $112.52 \pm 1.41\%$ . Therefore, nutritional and antioxidant properties of *K. alvarezii* varied according to strains and seasons, with the best result in the spring of 2017. In summer and autumn of 2017, the green strain had better nutritional and antioxidant profiles, whereas in the winter of 2017 and spring of 2017 it was the G11 strain and in the summer of 2018 it was the red strain.

**Keywords** Algae, Biological activity · Mineral composition, Phenolic compounds · Seaweed · Sulfated polysaccharides

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## Introduction

Seaweeds are one of the living renewable resources from the marine environment with potential for food and both therapeutic and biotechnological applications (Circuncisão et al. 2018; Oliveira et al. 2019; Shannon and Abu-Ghanam 2019; Muñoz and Díaz 2020). As a consequence of increasing demand, edible macroalgae species have been successfully produced in farm systems, such as the brown macroalgae *Undaria pinnatifida* (Harvey) Suringar, *Saccharina japonica* (Areschoug) C.E. Lane, C. Mayes, Druehl and G.W. Saunders, and the red macroalgae *Porphyra* C. Agardh/*Pyropia* J. Agardh, *Gracilaria* Greville, and *Kappaphycus* Doty/*Eucheuma* J. Agardh (Buschmann et al. 2017).

Currently, *Kappaphycus alvarezii* (Doty) Doty ex P.C. Silva and *Eucheuma* spp. lead the rankings for global volume of produced farmed seaweeds with reports of well-established commercial farms in almost 30 tropical and subtropical countries (Alemañ et al. 2019; Hurtado et al. 2019). The major application of *K. alvarezii* is still for carrageenan

production, a hydrocolloid extensively used in the food, pharmaceutical, and textile industries (Hurtado et al. 2015). However, new applications have been proposed based on recent studies and the knowledge of chemical and nutritional profiles, as well as the biological activities of this species (Shannon and Abu-Ghannam 2019; Kumar et al. 2021).

*Kappaphycus alvarezii* is an important source of nutritional compounds, such as carbohydrates, dietary fiber, proteins, vitamins (A, B1, B12, C, and D), minerals (Ca, P, Na, K, Fe, and I), and unsaturated and saturated fatty acids (Fayaz et al. 2005; Rajasulochana et al. 2010; Kumar et al. 2014; Ariano et al. 2021), encouraging their use in human and animal nutrition. Diverse components with antioxidant, antibacterial, antidiabetic, and anticancer properties are reported for *K. alvarezii* (Chew et al. 2008; Farah Diyana et al. 2015; Kanatt et al. 2015; Suganya et al. 2016; Araújo et al. 2020; Farah Nurshahida et al. 2020 et al. 2020), supporting the application of this seaweed in nutraceutical and pharmaceutical industries. Carrageenan has also been employed in bioactive packaging films with antioxidant properties used by the food industry (Kanatt et al. 2015) and as useful excipients in drug delivery systems for oral administration in the pharmaceutical sector (Ghanbarzadeh et al. 2018).

In addition, a couple of studies have demonstrated the efficacy of *K. alvarezii* extract as biofertilizers or source of potassium, biostimulants for diverse cultures (Karthikeyan and Shanmugam 2017; Gelli et al. 2020). The residues of this macroalga can also be used to produce bioethanol and hydrogen in the biorefinery context (Masarin et al. 2016; Cedeno et al. 2018; Rodrigues et al. 2019; Fonseca et al. 2020).

When considering better utilization of *K. alvarezii*, it is very important to understand the variations in both chemical and nutritional profiles of the seaweed as a result of seasonal fluctuation and diversity of strains. The differences in physiological and nutritional profiles of *K. alvarezii* strains may be related to differences in pigment contents and other metabolites, causing nutritional variations. Despite the lower growth rate of the G11 strain, when compared to the green, red, and brown strains, Hayashi et al. (2007) demonstrated that it had the best yield and quality of carrageenan. Adharini et al. (2020) reported differences in the nutritional composition of red and green strains of *K. alvarezii* farmed in Indonesia. The red strain had high levels of ash, fat, proteins, carbohydrates, vitamin C, calcium, and iron, while the green strain was rich in crude fiber, sodium, and water. Araújo et al. (2020) concluded that the green strain had the best antioxidant potential compared to other strains.

Studies have demonstrated variations in proteins, carbohydrates, fiber, and ash contents of *K. alvarezii* from

fluctuations of environmental parameters, such as seawater temperature, salinity, and available nutrients (Hayashi et al. 2011; Kumar et al. 2015; Adharini et al. 2020). Maximum contents of carbohydrates in *K. alvarezii* correlated with higher values of water temperature, salinity, and sunlight intensity, confirming the influence of these parameters on carbohydrate synthesis (Kumar et al. 2015).

*Kappaphycus alvarezii* was introduced in Brazil in 1995, in São Paulo State, southeastern coast (Paula et al. 2002), followed by other introductions along the Brazilian coast, such as Rio de Janeiro (Castelar et al. 2009), Santa Catarina (Hayashi et al. 2011), Paraíba (Araújo et al. 2013), and Pernambuco, Ceará, and Bahia (Torrano-Silva et al. 2010). The strains growing in São Paulo State are tetrasporophytes color green, red, and brown, and a gametophyte G11 strain (Édison José de Paula strain), originated from spores of the brown strains that have a different characteristic of growth rates, carrageen properties, yield, and antioxidant potential compared to the others strains of *K. alvarezii* (Araujo et al. 2020). Despite suitable commercial and environmental conditions for *K. alvarezii* production (Bulboa and Paula 2005; Hayashi et al. 2007, 2011; Castelar et al. 2009; Góes and Reis 2012; Araújo et al. 2020), the only large-scale commercial cultivation of this species on the Brazilian coast was ended in 2012, likely owing to poor profitability (Hayashi et al. 2017). Currently, *K. alvarezii* production in Brazil operates on a small scale, but even so, Brazil is the closest country among Latin American countries implementing commercial cultivation of this species (Hurtado et al. 2019).

In this context, diverse studies have been developed in recent years aimed at improving the profitability and application of *K. alvarezii* production in Brazil. Euchematoid seaweed produced on the São Paulo coast had a potential application for bioethanol production and hydrogen (Masarin et al. 2016; Roldán et al. 2017; Solorzano-Chavez et al. 2019; Fonseca et al. 2020), as agricultural biofertilizer (Gelli et al. 2020) and as a natural antioxidant source for human food and animal feed (Araújo et al. 2020). In addition, these reports demonstrated significant differences in chemical composition among the strains, suggesting different potential applications for each strain. For instance, Masarin et al. (2016) observed differences in carbohydrate and sulfate group levels, and Araújo et al. (2020) reported the green strain as the best antioxidant source.

Therefore, this study evaluated the nutritional profile and antioxidant properties of four strains of *K. alvarezii* (green, red, brown, and G11) cultivated on São Paulo coastline, southeastern of Brazil, in different seasons to improve the knowledge of chemical properties and promote the use diversification of the different *K. alvarezii* strains, as well as to identify most productive seasons.

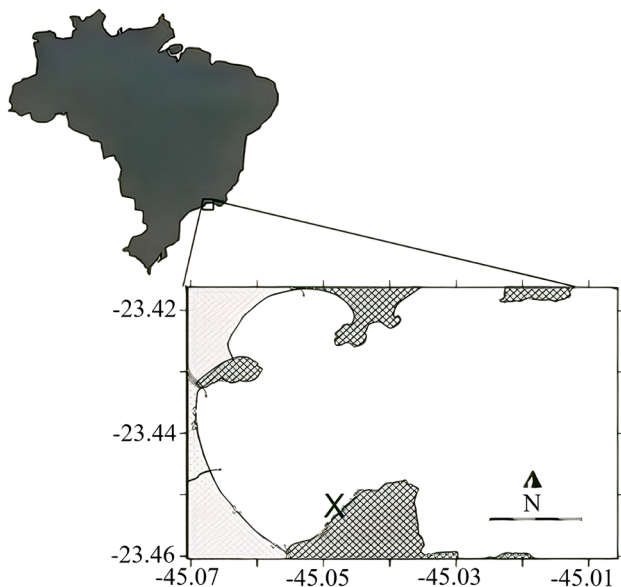
## Material and methods

### Algal collection

Three specimens of each strain (green, red, brown, and G11) ( $n = 12$ ) of *Kappaphycus alvarezii* were collected from a pilot cultivation of the Fisheries Institute located at Ubatuba Bay, on the north coast of São Paulo ( $23^{\circ} 27.134' \text{ S}$ ,  $45^{\circ} 02.817' \text{ W}$ ) (Fig. 1). The collections were performed once for seasons: summer (January), autumn (April), winter (July), and spring (November) of 2017 and summer (January) of 2018. The collected material was washed in tap water until all salt, sand, and epibionts had been removed and then over-dried at  $45^{\circ} \text{C}$  until obtaining constant weight. The dried algal samples were ground to a fine powder.

### Environmental parameters

The temperature, salinity, and transparency of seawater were recorded in situ near the cultivation raft of *K. alvarezii* every day from January 2017 to January 2018. Temperature was determined by an alcohol thermometer, salinity with a handheld refractometer, and transparency with a Secchi disk. Data of seawater nutrients in Ubatuba Bay were obtained from Aidar et al. (1993) and precipitation data were obtained from the site <https://giovanni.gsfc.nasa.gov/giovanni/>.



**Fig. 1** Location of cultivation of *Kappaphycus alvarezii* at Ubatuba Bay, Southeastern Brazilian coast

### Ash and minerals

The ash content was determined thermogravimetrically by ashing at  $550^{\circ} \text{C}$  until a constant weight was attained according to AOAC method 968.06 (1990). The samples were ashed according to DIN EN ISO 14891 at  $550^{\circ} \text{C}$  and  $950^{\circ} \text{C}$  until complete incineration.

Samples of 250 mg dry weight (DW) of each strain and season ( $n = 2$ ) were digested in concentrated citric acid and 30% hydrogen peroxide in thermic digestion blocks (DigiPrep, SCP Science, USA), and mineral contents (Ca, K, Mg, Na, and Fe) were determined by the inductively coupled plasma optical emission spectroscopy method (ICP-OES, Arcos, USA).

### Soluble carbohydrates

Soluble carbohydrate content was quantified by the phenol-sulfuric acid method described by Masuko et al. (2005). Samples of 165 mg DW of each strain and season ( $n = 3$ ) were extracted with 1 mL of ultrapure water at  $70^{\circ} \text{C}$  for 3 h. Soon after the sample was centrifuged at 12,000 rpm for 10 min at room temperature. The supernatant was collected, and the concentration of soluble carbohydrate was determined by spectrophotometry using a 96-well microplate. Galactose was used as the reference substance to determine the standard curve ( $15\text{--}75 \mu\text{g mL}^{-1}$ ;  $y = 0.0495x - 0.1379$ ;  $R^2 = 0.9949$ ).

### Proteins and amino acids

Protein content was analyzed according to the Dumas combustion method (Wiles et al. 1998) through the measurement of nitrogen content using the FP-528 Nitrogen/Protein Determinator (Leco Corporation, USA). Protein content was determined according to the conversion equation ( $N$ )  $\times 6.25$  (ISO 16634-1 2008).

Amino acid profile was determined according to Santa-Catarina et al. (2006) and modified by Urrea-Victoria et al. (2020). Samples of 100 mg DW of each strain and season ( $n = 3$ ) were extracted with 5 mL of 80% ethanol for 2 h. Afterwards, the supernatant was collected, centrifuged at 12,000 rpm for 10 min at room temperature, and dried in speed-vacuum at  $45^{\circ} \text{C}$ . The concentrate was resuspended in 1.5 mL of ultrapure water, centrifuged at 12,000 rpm for 10 min at room temperature, and filtrated with a  $0.2\text{-}\mu\text{m}$  Millipore membrane. Samples were derivatized with an o-phthaldialdehyde (OPA) solution and analyzed by HPLC (Shimadzu Shin-pack CLC ODS) using a C18 reverse phase column (Supelcosil LC-18,  $25 \text{ cm} \times 4.6 \text{ mm L}^{-1} \times \text{i.d.}$ ) with a gradient of 65% methanol following Egydio et al. (2013) in a buffer solution (50 mM sodium acetate, 50 mM sodium phosphate,  $20 \text{ mL L}^{-1}$  methanol,  $20 \text{ mL L}^{-1}$  tetrahydrofuran, and pH 8.1 adjusted with acetic acid). Amino acids were detected by fluorescence excitation in wavelengths of

250 nm and emission in 480 nm. Peak areas and retention times were determined by comparison to measures of standard amino acids (Sigma-Aldrich, USA).

## Antioxidant potential

**Extract preparation** Samples of dried and powdered mass (approximately 300 g DW) were macerated with five different solvents in increasing order of polarity: hexane, dichloromethane, ethyl acetate, methanol, and hot water, in a sequential extraction at a proportion of 1 g of sample and 30 mL of solvent. The maceration procedure for each solvent ( $n=5$ ) was performed for 72 h at room temperature with the replacement of solvent every 24 h, except for aqueous extraction, which was carried out at 80 °C, replacing the water every 3 h. Soon after, the extracts of each solvent were filtered, picked up, and lyophilized, and the crude extract yield was determined.

**Antioxidant assays** The antioxidant potential of *K. alvarezii* was determined for the methanolic extract ( $n=5$ ) at a concentration of 3 mg mL<sup>-1</sup> diluted in 10% DMSO. Four in vitro antioxidant assays were conducted: Folin-Ciocalteu reducing capacity (commonly used for phenolic compound quantification), DPPH and ABTS radical scavenging, and ferric reducing antioxidant power (FRAP) performed as described by Araújo et al. (2020). All absorbance measurements were carried out in a 96-well microplate UV/vis spectrophotometer (Epoch, Biotek, USA) at 760 nm for Folin-Ciocalteu assay, 517 nm for DPPH assay, 734 nm for ABTS, and 595 nm for FRAP assay (Araújo et al. 2020). Gallic acid was used as a reference substance for the standard curve (Table 1). The results were expressed as a percentage of antioxidant activity, and only total phenolic content (TPC) was expressed as gallic acid equivalent (mg GAE g<sup>-1</sup> DW).

## Composite index

A composite index integrating contents of carbohydrates, minerals, amino acids, and total antioxidant potential was calculated by giving each parameter an equal weight with an index value of 100 to the best score for each descriptor. Next, the means of the individual score index were ranked in decreasing order by color strain.

Composite index score = [(sample score/best score) × 100].

## Statistical analyses

All data are expressed as mean ± standard deviation. Analysis of one-way ANOVA and factorial ANOVA was conducted to determine significant differences among the four strains and seasonal variation of mineral content, carbohydrates, and amino acids, as well as antioxidant activities of *K. alvarezii*. Then, the post hoc multiple comparison Newman-Keuls test at 95% significance level ( $p < 0.05$ ) was used to identify significant differences among the sample means. The relationships among descriptors (total minerals, carbohydrates, and amino acids) and environmental data (seawater temperature, salinity, and transparency) were evaluated by Spearman's correlation coefficients.

## Results

### Environmental parameters

Mean surface seawater temperature in the area near *K. alvarezii* cultivation varied from 22.7 to 31.5 °C, salinity varied from 34 to 37 psu, and seawater transparency was up to 3 m in depth (Fig. 2). Precipitation at Ubatuba Bay varied from 225.00 mm month<sup>-1</sup> during summer to 20.00 mm month<sup>-1</sup> during winter (Fig. 2). In relation to nutrient levels at Ubatuba Bay, Aidar et al. (1993) reported a tendency of lower concentrations of nitrite, nitrate, silicate, and phosphate in summer than in winter, and lower ammonium levels during winter than during summer (Fig. 3).

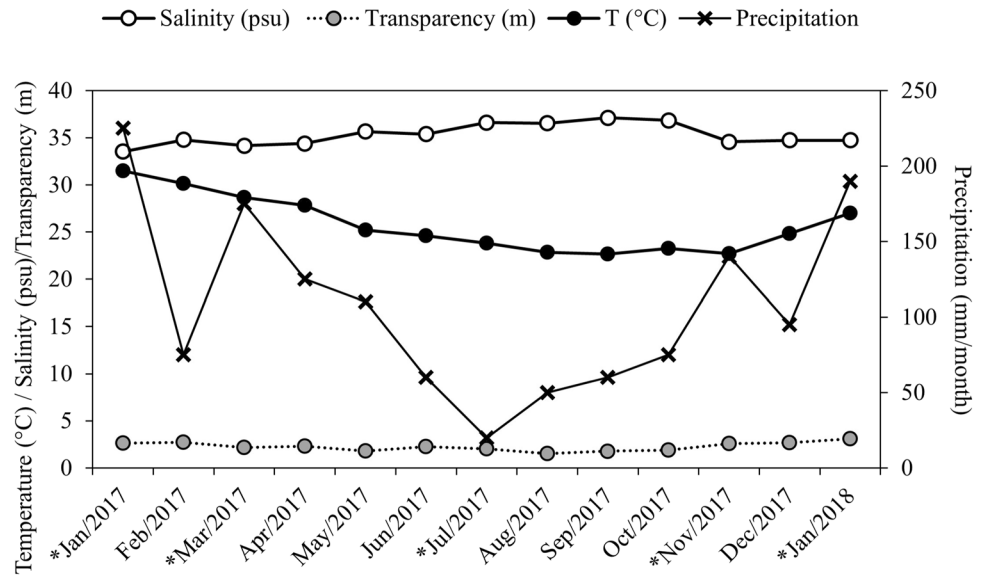
### Ash and minerals

The ash content of *K. alvarezii* ranged 11.65 to 25.60%. The G11 strain had the highest percentage of ash with 25.60%, followed by green (14.54%), brown (14.10%), and red (11.65%). Total mineral content of *K. alvarezii* by season ranged from 10,130.90 ± 1,613.78 mg (100 g)<sup>-1</sup> DW in the summer of 2018 to 12,561.20 ± 2,190.72 mg (100 g)<sup>-1</sup> DW in the summer of 2017 (Table 2). Total mineral content

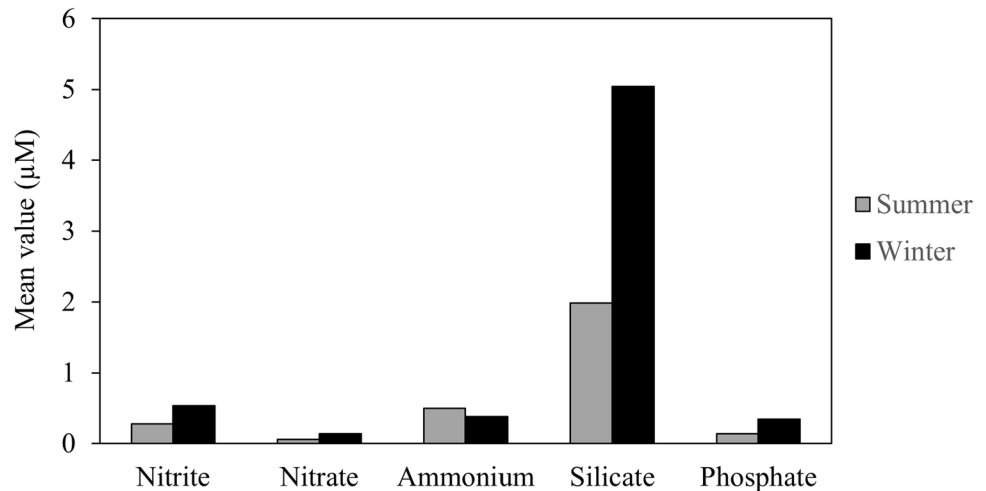
**Table 1** Parameters of the standard curves of gallic acid for different antioxidant assays

Antioxidant assay	Concentration (µg mL <sup>-1</sup> )	Linear equation (y = ax + b)	Regression coefficient (R <sup>2</sup> )
Folin-Ciocalteu	2–10	y = 0.1629x + 0.0762	0.9846
DPPH	0.5–2.5	y = -0.6869x + 1.0773	0.9928
ABTS	0.5–3.3	y = -0.9635x + 0.8316	0.9879
FRAP	0.5–3.4	y = 1.5069x + 0.1052	0.9961

**Fig. 2** Environmental data (surface temperature, salinity, transparency of seawater, and precipitation) collected near the *Kappaphycus alvarezii* cultivation. Asterisks show the months that seaweeds were collected for analyses



**Fig. 3** Mean value of nutrients (nitrite, nitrate, ammonium, silicate, and phosphate) at Ubatuba Bay from 1985 to 1988, according to Aidar et al. (1993)



**Table 2** The average of mineral ( $\text{mg } (100 \text{ g})^{-1} \text{ DW}$ ) and amino acid ( $\mu\text{g g}^{-1} \text{ DW}$ ) contents of *K. alvarezii* by season and recommended intake of mineral values per day (Otten et al. 2016). Values are mean  $\pm$  sd

	Summer 17	Autumn 17	Winter 17	Spring 17	Summer 18	Recommended intake values per day ( $\text{mg day}^{-1}$ )
<b>Minerals</b>						
Fe	$0.72 \pm 1.45^a$	$8.39 \pm 2.15^b$	$3.25 \pm 1.24^a$	$6.76 \pm 1.65^b$	$8.40 \pm 2.07^b$	11.5
Ca	$197.25 \pm 6.73$	$187.25 \pm 13.69$	$183 \pm 55.37$	$163.5 \pm 35.02$	$201 \pm 24.75$	1.10
Mg	$460.12 \pm 49.46^b$	$397.25 \pm 50.17^{ab}$	$355.87 \pm 105.82^a$	$315.37 \pm 99.45^a$	$365.12 \pm 36.92^{ab}$	326
Na	$2,154.87 \pm 124.14^B$	$1,629.37 \pm 397.79^A$	$1,572.25 \pm 192.07^A$	$1,592.75 \pm 413.49^A$	$1,818 \pm 123.56^{AB}$	1,500
K	$9,748.25 \pm 2,290.99$	$8,152 \pm 955.06$	$8,867.87 \pm 2,491.50$	$8,371.5 \pm 448.61$	$7,738.37 \pm 1504.90$	4,700
Total minerals	$12,561.20 \pm 2,190.72^A$	$10,374.30 \pm 1,373.56^B$	$10,982.30 \pm 2,239.04^A$	$10,449.90 \pm 237.19^A$	$10,130.90 \pm 1613.78^B$	
Amino acids	$586.00 \pm 462.76^{bc}$	$106.91 \pm 46.03^a$	$190.88 \pm 121.55^{ab}$	$384.23 \pm 326.45^{abc}$	$743.70 \pm 625.33^c$	

Superscript letters derived from post hoc Newman–Keuls after ANOVA test ( $P < 0.05$ ) indicate significant differences among seasons

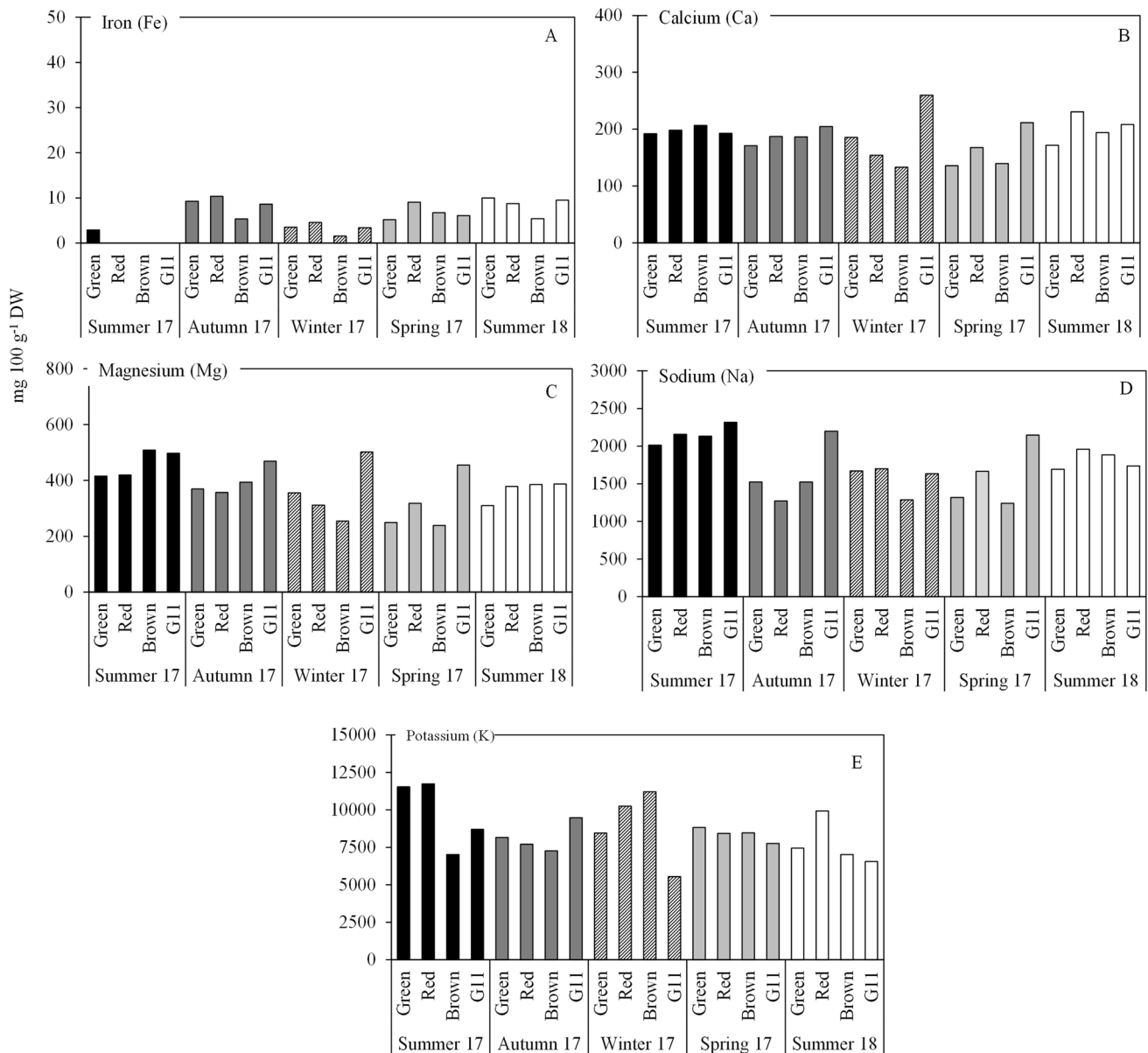
\*Significance for Fe ( $F = 29.29$ ,  $p < 0.05$ ), Mg ( $F = 4.86$ ,  $p < 0.05$ ), Na ( $F = 6.65$ ,  $p < 0.05$ ), and total minerals ( $F = 7.67$ ,  $p < 0.05$ )



of *K. alvarezii* had fluctuation significant among seasons (one-way ANOVA,  $F=2.98$ ,  $p<0.05$ ), and Fe levels (one-way ANOVA,  $F=29.29$ ,  $p<0.05$ ), Mg (one-way ANOVA,  $F=4.86$ ,  $p<0.05$ ), and Na (one-way ANOVA,  $F=6.65$ ,  $p<0.05$ ) fluctuated by season.

Fe, Ca, Mg, Na, and K contents by season and strain are shown in Fig. 4. The average Fe content was  $5.50 \text{ mg } (100 \text{ g})^{-1} \text{ DW}$ , representing the lowest level among the analyzed minerals, which ranged from  $0 \pm 0$  to  $10.36 \pm 2.22 \text{ mg } (100 \text{ g})^{-1} \text{ DW}$  (Fig. 4A). The highest Fe contents were observed in autumn 2017, spring 2017, and summer 2018 in the green, red, and G11 strains. Ca level ranged from

$133.00 \pm 2.82$  to  $259.50 \pm 7.77 \text{ mg } (100 \text{ g})^{-1} \text{ DW}$  (Fig. 4B) with the highest value of Ca occurring in the G11 strain during winter 2017. Mg amount fluctuated from  $239.00 \pm 2.82$  to  $508.50 \pm 13.43 \text{ mg } (100 \text{ g})^{-1} \text{ DW}$  (Fig. 4C) with the highest levels during summer 2017 and autumn 2017, especially for the brown and G11 strains. Na level varied from  $1241.00 \pm 2.82$  to  $2315.00 \pm 93.33 \text{ mg } (100 \text{ g})^{-1} \text{ DW}$  (Fig. 4D) with the highest values in the brown and G11 strains. K content ranged from  $5,545.00 \pm 137.17$  to  $11,742.00 \pm 377.99 \text{ mg } (100 \text{ g})^{-1} \text{ DW}$  (Fig. 4E) with significant variation according to season and strain. It is important to note that K accounted for the highest mineral level.



**Fig. 4** Seasonal variation of mineral content of four strains of *Kappaphycus alvarezii*. Contents of (A) iron (Fe), (B) calcium (Ca), (C) magnesium (Mg), (D) sodium (Na), and (E) potassium (K)

Cadmium and Cu contents were also measured, and lower values ( $<0.05$  ppm) were registered below the maximum allowed concentration limit for food.

### Soluble carbohydrates

Carbohydrate content of *K. alvarezii* varied according to season and strain (factorial ANOVA,  $F = 3.98$ ,  $p < 0.05$ ; Fig. 5), and a significant negative correlation of carbohydrate content and seawater temperature ( $r = -0.60$ ,  $p < 0.05$ , Table 3) was observed.

The lowest total content of carbohydrate occurred in summer 2017 at  $122.92 \pm 15.11$  mg g<sup>-1</sup> DW, and the highest amount appeared in summer 2018 at  $231.79 \pm 16.86$  mg g<sup>-1</sup> DW (Fig. 5). The contents of carbohydrates by strain also varied by season. The carbohydrate level of the green strain ranged from  $108.48 \pm 30.13$  to  $208.74 \pm 37.09$  mg g<sup>-1</sup> DW with the highest values in winter 2017, spring 2017, and summer 2018. Carbohydrate content in the red strain was from  $170.52 \pm 13.67$  to  $260.56 \pm 4.24$  mg g<sup>-1</sup> DW with the highest levels in summer 2018. Carbohydrate contents in the brown strain varied from  $99.99 \pm 8.05$  to  $247.27 \pm 10.02$  mg g<sup>-1</sup> DW with the best values in autumn 2017, and in the G11 strain, carbohydrates ranged from  $109.22 \pm 6.65$  to  $259.01 \pm 1.57$  mg g<sup>-1</sup> DW in winter 2017.

### Proteins and amino acids

The mean of protein content of *K. alvarezii* was  $7.59 \pm 0.92$  mg (100 g)<sup>-1</sup> DW. The highest protein value was observed in the brown strain ( $8.79$  mg (100 g)<sup>-1</sup> DW), followed by red ( $7.84$  mg (100 g)<sup>-1</sup> DW), G11 ( $6.90$  mg (100 g)<sup>-1</sup> DW), and green ( $6.84$  mg (100 g)<sup>-1</sup> DW).

**Table 3** Spearman correlation coefficients ( $n=3$ ) of carbohydrates, total minerals, and amino acids of *Kappaphycus alvarezii* and environmental data

	Strain	Season	Temperature	Salinity	Transparency
Carbohydrate	0.14	<b>0.63*</b>	<b>-0.60*</b>	<b>0.66*</b>	-0.13
Total minerals	-0.38	-0.14	0.01	0.00	-0.04
Amino acid	0.18	-0.10	0.00	-0.15	-0.04

Bold values indicate significant correlations ( $r = -0.60$ ,  $p < 0.05$ )

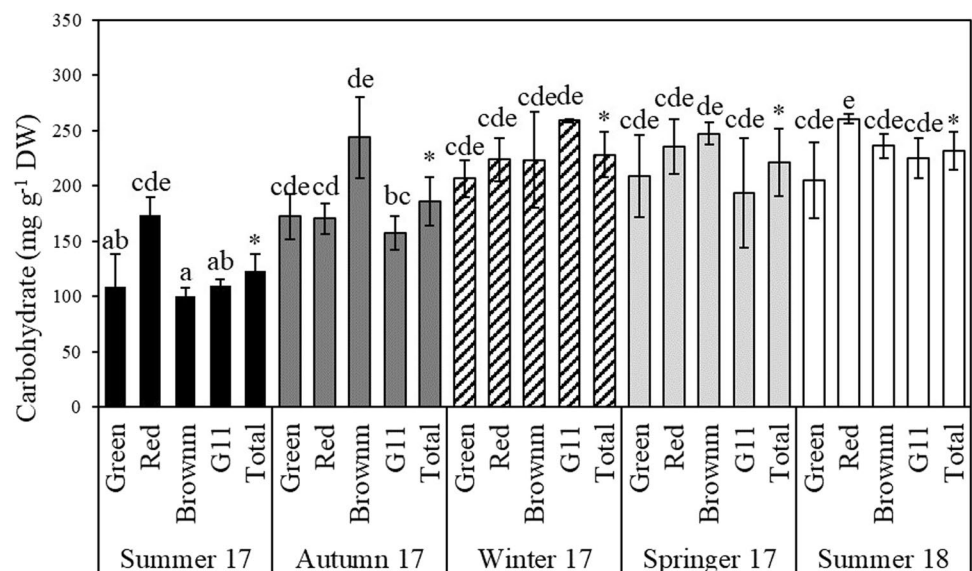
\*Significance ( $r = -0.60$ ,  $p < 0.05$ )

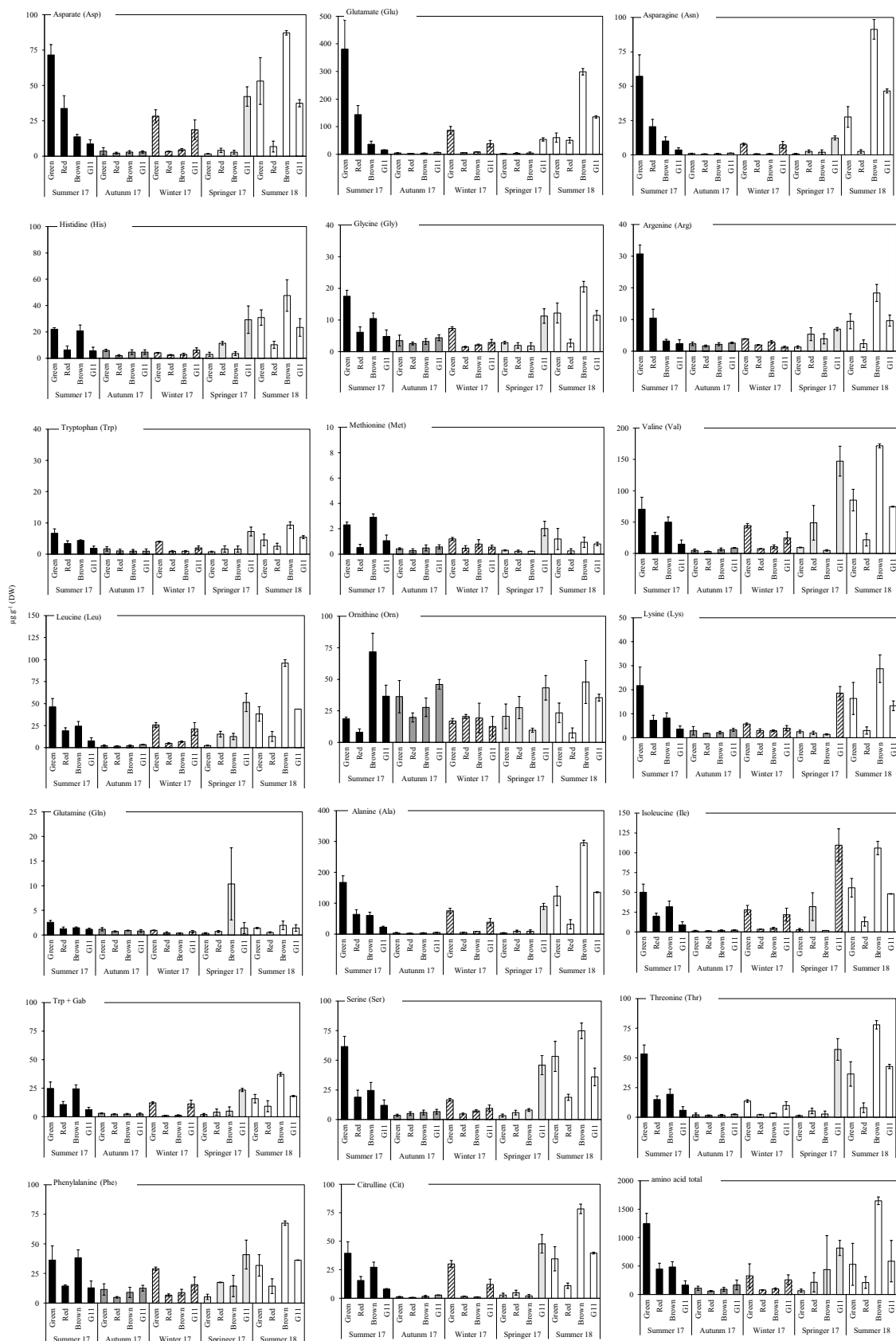
The average total content of free amino acids ranged seasonally (one-way ANOVA,  $F = 5.70$ ,  $p < 0.05$ ), with the lowest value during autumn 2017 ( $106.91 \pm 46.03$ ) and the highest values in the summer 2017 and 2018 ( $586.00 \pm 462.76$  and  $743.70 \pm 625.33$  µg g<sup>-1</sup> DW, respectively) (Table 2). Twenty amino acids were identified, of which glutamic acid (GLU) and alanine (ALA) were the main constituents (Fig. 6).

### Antioxidant potential

The total phenolic content (TPC) of *K. alvarezii* was assessed by Folin-Ciocalteu assay. Average of TPC ranged seasonally, with the lowest values during summer 17 ( $46.26 \pm 112.52$  mg GAE g<sup>-1</sup> DW) and the highest in the autumn 17 ( $101.39 \pm 24.73$  mg GAE g<sup>-1</sup> DW) (one-way ANOVA,  $F = 51.04$ ,  $p = 0.00$ , Tables 4 and 5). Statistical differences of TPC content were observed among strains and seasons (factorial ANOVA,  $F = 8.58$ ,  $p = 0.00$ , Table 5) with the highest TPC levels occurring in autumn 2017.

**Fig. 5** Seasonal variation of carbohydrate content of *Kappaphycus alvarezii*. Asterisks indicate total carbohydrate content, and letters indicate the differences between strains and seasons as post hoc Newman-Keuls after factorial ANOVA ( $p < 0.05$ )





**Fig. 6** Seasonal variation of amino acid content of strains of *Kappaphycus alvarezii*



TPC content was also analyzed as percentage of antioxidant activity (% AOX), which ranged from  $78.83 \pm 3.02$  to  $96.44 \pm 0.34\%$  (Fig. 7A). Significant differences in antioxidant activity percentages were also observed (factorial ANOVA,  $F = 16.51$ ,  $p = 0.00$ ; Fig. 7A) with the highest values occurring in autumn 2017 in the red strain and in summer 18 in all strains.

DPPH radical-scavenging activity ranged from  $13.29 \pm 1.20$  to  $61.07 \pm 3.43\%$  (Fig. 7B) with significant differences within strains and seasons (factorial ANOVA,  $F = 2.03$ ;  $p < 0.05$ ; Fig. 7B). The smallest antioxidant potential occurred in summer 2017. Ferric reducing antioxidant potential (FRAP) ranged from  $58.73 \pm 3.96$  to  $102.56 \pm 3.09\%$  (Fig. 7C). Statistical differences in FRAP antioxidant activity were observed according to seasons and strains (factorial ANOVA,  $F = 1.23$ ;  $p < 0.05$ ; Fig. 7C) with the lowest value occurring in summer 2017. ABTS radical-scavenging potential (ABTS) varied from  $95.29 \pm 4.31$  to  $112.52 \pm 1.41\%$  (Fig. 7D). Despite differences in ABTS activity by analyzed strains and seasons (factorial ANOVA,  $F = 15.00$ ,  $p < 0.05$ ; Fig. 7D), these disparities were minimal.

The composite index was used to weight and order the nutritional and antioxidant potential of *K. alvarezii*, considering strain and season. The best nutritional and antioxidant potential of *K. alvarezii* was in spring 2017 (Table 6). The green strain had the highest nutritional and antioxidant potential compared to the other strains in summer 2017 and autumn 2017. During winter 2017 and spring 2017, the best strain was G11, and in summer 2018, it was the red (Table 6).

## Discussion

Seaweeds have significant nutrient contents and biological activities, which can be used for the development of nutraceutical products for human and animal health and nutrition, following a growing trend of consumption of natural products in addition to the increasing added value of this natural resource (Ariano et al. 2021). These nutritional profiles and biological activities of seaweed can fluctuate according to biological diversity and geographical and environmental factors, such as light and nutrients available, water temperature, and salinity (Hayashi et al. 2007; Adharini et al. 2020). Consequently, it is equally important to be aware of the variants or strains of seaweeds farmed and understand how environmental conditions influence the nutritional contents of these macroalgae.

The ash content of seaweed is typically used to estimate its mineral content. Macroalgae are known for their higher mineral content compared to land vegetables, making them promising organisms for developing nutraceutical products (Rupérez 2002; Cotas et al. 2020). This mineral abundance is related to availability in seawater as a consequence of

specificity, geography, and environmental conditions (Kumar et al. 2015). In the present study, the ash content of *K. alvarezii* represented up to 25% of samples analyzed, indicating the significant mineral amount in this seaweed. The average content of Ca, Mg, Na, and K estimated in *K. alvarezii* was equivalent to Dietary Reference Intakes (DRI) (Table 2) developed by the U.S. Institute of Medicine and Health Canada (Otten et al. 2016), which has been used worldwide.

Fe content ( $0 \pm 0$  to  $10.36 \pm 2.22$  mg  $(100 \text{ g})^{-1}$  DW) was the lowest among minerals analyzed in this study when compared to other studies reported for *K. alvarezii* farmed in India with 65.95 to 789 mg  $(100 \text{ g})^{-1}$  DW (Rajasulochana et al. 2010; Kumar et al. 2015), Malaysia with 4000 mg  $(100 \text{ g})^{-1}$  DW (Ariano et al. 2021), and Indonesia with 24 to 30 mg  $(100 \text{ g})^{-1}$  DW (Adharini et al. 2020). The difference in mineral contents for *K. alvarezii* cultivated at different regions may be related to variations of environmental parameters specific from the local like nutrient availability. For example, seawater temperature and salinity of the Indian coast reported by Kumar et al. (2015) on *K. alvarezii* cultivation were similar to those observed in the São Paulo coast. Other reports of Fe contents in other edible seaweeds are 183 mg  $(100 \text{ g})^{-1}$  DW for *Neopyropia tenera* (Kjellman) L.-E. Yang & J. Brodie (as *Porphyra tenera*), 5.2 mg  $(100 \text{ g})^{-1}$  DW for *Porphyra umbilicalis* Kützinger, 71 mg  $(100 \text{ g})^{-1}$  DW for *Palmaria palmata* (Linnaeus) F. Weber & D. Mohr, and 3.9 mg  $(100 \text{ g})^{-1}$  DW *Undaria pinnatifida* (Harvey) Suringar (MacArtain et al. 2007; Muñoz and Díaz 2020).

Ca and Mg contents of *K. alvarezii* studied had significant mean levels with 186.4 mg  $(100 \text{ g})^{-1}$  DW and 378 mg  $(100 \text{ g})^{-1}$  DW, respectively. Ca and Mg levels of *K. alvarezii* farmed on the coastline of São Paulo, Brazil, were better than values reported for *K. alvarezii* cultivated in others countries (Ca—76.25 to 459.23 mg  $(100 \text{ g})^{-1}$  DW; Mg—266.45 mg  $(100 \text{ g})^{-1}$  DW), *Porphyra umbilicalis* (Ca—34.2 mg  $(100 \text{ g})^{-1}$  DW; Mg—108.3 mg  $(100 \text{ g})^{-1}$  DW), *Palmaria palmata* (Mg—266.45 mg  $(100 \text{ g})^{-1}$  DW), *Undaria pinnatifida* (Ca—112.3 mg  $(100 \text{ g})^{-1}$  DW; Mg—78.7 mg  $(100 \text{ g})^{-1}$  DW), and *Laminaria digitata* (Hudson) J.V. Lamouroux (Ca—1005 mg  $(100 \text{ g})^{-1}$  DW) (MacArtain et al. 2007; Adharini et al. 2020; Alcantara and Lazaro-Llanos 2020; Muñoz and Díaz 2020; Ariano et al. 2021). Concerning heavy metals analyzed, Cd and Cu contents were much lower than the toxic limits permitted for human consumption (Kumar et al. 2015), ensuring the safe intake of this seaweed. It is fundamental to reinforce the need to systematically assess the heavy metal content of seaweed farmed or collected for human food.

Although seasonal fluctuation of mineral contents of *K. alvarezii* has been detected, this variation was minor; consequently, no specific season was noted for mineral consumption. Therefore, we can endorse the use of *K. alvarezii* as a mineral source in any season. The four strains of *K.*

**Table 4** Amino acid content ( $\mu\text{g g}^{-1}$  DW) of four strains of *Kappaphycus alvarezii* in all seasons. Values are mean ( $n=3$ )  $\pm$  sd

Strains	Season	ASP	GLU	ASN	SER	GLN	HIS	GLY	ARG	TRE	ALA	TRP
Green	Summer 17	71.55 $\pm$ 7.39	381.05 $\pm$ 105.53	57.20 $\pm$ 15.61	61.60 $\pm$ 8.60	2.58 $\pm$ 0.38	22.08 $\pm$ 1.65	17.51 $\pm$ 1.87	30.69 $\pm$ 2.80	53.35 $\pm$ 7.58	167.62 $\pm$ 21.42	6.69 $\pm$ 1.43
	Autumn 17	3.60 $\pm$ 2.49	4.84 $\pm$ 1.09	1.00 $\pm$ 0.22	3.52 $\pm$ 0.95	1.16 $\pm$ 0.33	5.99 $\pm$ 0.30	3.50 $\pm$ 1.71	2.30 $\pm$ 0.55	1.96 $\pm$ 1.57	3.41 $\pm$ 2.40	1.69 $\pm$ 0.72
	Winter 17	28.25 $\pm$ 4.60	86.93 $\pm$ 13.80	7.85 $\pm$ 0.70	16.64 $\pm$ 1.17	0.95 $\pm$ 0.06	4.21 $\pm$ 0.02	7.33 $\pm$ 0.56	3.88 $\pm$ 0.08	13.66 $\pm$ 1.11	75.53 $\pm$ 8.36	4.03 $\pm$ 0.14
	Spring 17	1.64 $\pm$ 0.25	3.10 $\pm$ 0.03	0.70 $\pm$ 0.40	3.29 $\pm$ 1.30	0.36 $\pm$ 0.11	3.05 $\pm$ 1.59	2.84 $\pm$ 0.43	1.31 $\pm$ 0.37	1.30 $\pm$ 0.46	3.75 $\pm$ 1.29	0.76 $\pm$ 0.20
	Summer 18	53.11 $\pm$ 16.54	60.46 $\pm$ 16.85	27.60 $\pm$ 7.54	53.28 $\pm$ 12.68	1.41 $\pm$ 0.13	30.97 $\pm$ 5.85	12.20 $\pm$ 3.17	9.45 $\pm$ 2.38	36.43 $\pm$ 10.31	123.42 $\pm$ 31.11	4.55 $\pm$ 1.85
Red	Summer 17	33.76 $\pm$ 8.98	143.76 $\pm$ 32.94	20.54 $\pm$ 5.31	18.88 $\pm$ 5.90	1.26 $\pm$ 0.36	6.36 $\pm$ 2.94	6.14 $\pm$ 0.45	10.44 $\pm$ 2.82	14.88 $\pm$ 3.09	64.06 $\pm$ 15.18	3.40 $\pm$ 0.90
	Autumn 17	2.18 $\pm$ 0.67	2.81 $\pm$ 0.08	0.65 $\pm$ 0.17	5.01 $\pm$ 1.56	0.72 $\pm$ 0.12	2.04 $\pm$ 0.75	2.58 $\pm$ 0.45	1.62 $\pm$ 0.32	1.49 $\pm$ 0.45	3.09 $\pm$ 0.50	1.02 $\pm$ 0.60
	Winter 17	3.30 $\pm$ 0.26	5.96 $\pm$ 0.34	0.72 $\pm$ 0.14	4.67 $\pm$ 0.75	0.41 $\pm$ 0.24	2.56 $\pm$ 0.40	1.51 $\pm$ 0.19	1.90 $\pm$ 0.13	2.11 $\pm$ 0.32	5.67 $\pm$ 0.04	0.92 $\pm$ 0.23
	Spring 17	4.15 $\pm$ 1.53	4.10 $\pm$ 1.82	2.59 $\pm$ 1.09	5.83 $\pm$ 1.96	0.70 $\pm$ 0.19	11.48 $\pm$ 1.28	1.97 $\pm$ 0.87	5.39 $\pm$ 2.04	5.29 $\pm$ 2.30	9.32 $\pm$ 3.79	1.64 $\pm$ 1.01
	Summer 18	6.83 $\pm$ 3.87	50.79 $\pm$ 10.34	2.51 $\pm$ 1.26	18.73 $\pm$ 2.61	0.51 $\pm$ 0.14	10.30 $\pm$ 2.67	2.70 $\pm$ 1.22	2.36 $\pm$ 1.19	8.00 $\pm$ 4.10	31.96 $\pm$ 15.04	2.54 $\pm$ 0.97
Brown	Summer 17	13.70 $\pm$ 1.71	36.43 $\pm$ 11.24	10.11 $\pm$ 3.03	24.55 $\pm$ 6.74	1.46 $\pm$ 0.16	20.58 $\pm$ 4.73	10.46 $\pm$ 1.75	3.20 $\pm$ 0.55	19.34 $\pm$ 4.31	60.39 $\pm$ 10.76	4.38 $\pm$ 0.20
	Autumn 17	2.92 $\pm$ 1.04	4.11 $\pm$ 1.18	0.89 $\pm$ 0.26	5.91 $\pm$ 2.03	0.91 $\pm$ 0.09	4.72 $\pm$ 1.79	3.21 $\pm$ 0.90	2.20 $\pm$ 0.48	1.74 $\pm$ 0.67	3.65 $\pm$ 1.29	0.99 $\pm$ 0.51
	Winter 17	4.54 $\pm$ 0.86	8.28 $\pm$ 0.73	0.82 $\pm$ 0.11	7.25 $\pm$ 0.70	0.38 $\pm$ 0.07	2.91 $\pm$ 0.98	2.14 $\pm$ 0.24	2.91 $\pm$ 0.41	3.34 $\pm$ 0.16	8.80 $\pm$ 0.38	0.93 $\pm$ 0.22
	Spring 17	2.83 $\pm$ 1.30	4.31 $\pm$ 4.06	2.02 $\pm$ 1.63	8.00 $\pm$ 1.68	10.37 $\pm$ 0.10	3.59 $\pm$ 1.44	1.81 $\pm$ 1.03	3.91 $\pm$ 1.61	2.65 $\pm$ 2.54	9.22 $\pm$ 4.64	1.63 $\pm$ 0.98
	Summer 18	87.18 $\pm$ 1.57	299.02 $\pm$ 0.91	91.32 $\pm$ 7.13	74.87 $\pm$ 6.56	1.95 $\pm$ 0.88	47.65 $\pm$ 11.99	20.51 $\pm$ 1.70	18.38 $\pm$ 2.70	77.93 $\pm$ 3.51	295.70 $\pm$ 8.42	9.30 $\pm$ 1.08
G11	Summer 17	8.67 $\pm$ 2.94	15.93 $\pm$ 0.45	3.62 $\pm$ 1.53	11.97 $\pm$ 4.54	1.16 $\pm$ 0.21	5.48 $\pm$ 3.00	4.81 $\pm$ 2.04	2.30 $\pm$ 1.30	5.82 $\pm$ 3.05	22.57 $\pm$ 3.04	1.82 $\pm$ 0.75
	Autumn 17	3.01 $\pm$ 0.79	6.75 $\pm$ 0.91	1.27 $\pm$ 0.12	6.55 $\pm$ 2.04	0.82 $\pm$ 0.28	4.73 $\pm$ 1.72	4.39 $\pm$ 0.92	2.62 $\pm$ 0.23	2.43 $\pm$ 0.25	5.41 $\pm$ 0.08	0.95 $\pm$ 0.73
	Winter 17	18.68 $\pm$ 1.93	37.90 $\pm$ 12.38	7.30 $\pm$ 2.52	9.44 $\pm$ 2.71	0.68 $\pm$ 0.22	6.19 $\pm$ 1.77	2.89 $\pm$ 0.97	1.27 $\pm$ 0.27	9.86 $\pm$ 3.20	38.54 $\pm$ 12.11	1.99 $\pm$ 0.63
	Spring 17	42.18 $\pm$ 6.89	53.90 $\pm$ 6.06	12.35 $\pm$ 1.49	45.88 $\pm$ 8.06	1.40 $\pm$ 1.12	29.33 $\pm$ 10.43	11.24 $\pm$ 2.29	6.96 $\pm$ 0.51	57.14 $\pm$ 9.13	89.77 $\pm$ 9.97	7.24 $\pm$ 1.43
	Summer 18	37.44 $\pm$ 2.54	135.61 $\pm$ 4.36	46.49 $\pm$ 1.47	35.97 $\pm$ 7.46	1.42 $\pm$ 0.67	23.43 $\pm$ 6.67	11.46 $\pm$ 1.53	9.63 $\pm$ 1.75	42.76 $\pm$ 1.78	135.62 $\pm$ 1.63	5.45 $\pm$ 0.46
Strains	Season	MET	VAL	PHE	ILE	LEU	ORN	LIS	CIT	TIR+GABA	Total amino acid	
Green	Summer 17	2.31 $\pm$ 0.22	70.61 $\pm$ 19.09	36.6 $\pm$ 11.98	50.09 $\pm$ 10.32	46.33 $\pm$ 9.47	18.70 $\pm$ 1.28	21.73 $\pm$ 7.72	39.48 $\pm$ 10.01	24.80 $\pm$ 5.67	1,246.60 $\pm$ 180.63	
	Autumn 17	0.42 $\pm$ 0.08	4.96 $\pm$ 2.19	11.51 $\pm$ 4.82	1.45 $\pm$ 0.62	2.31 $\pm$ 0.85	36.21 $\pm$ 12.81	2.90 $\pm$ 1.76	1.25 $\pm$ 0.46	3.01 $\pm$ 0.46	108.06 $\pm$ 37.85	
	Winter 17	1.20 $\pm$ 0.12	44.31 $\pm$ 3.50	29.02 $\pm$ 1.44	27.76 $\pm$ 5.73	25.79 $\pm$ 2.76	16.92 $\pm$ 2.04	5.72 $\pm$ 0.44	30.12 $\pm$ 3.02	12.18 $\pm$ 0.89	328.05 $\pm$ 211.87	
	Spring 17	0.30 $\pm$ 0.05	9.45 $\pm$ 0.61	5.27 $\pm$ 2.23	2.83 $\pm$ 1.55	2.33 $\pm$ 0.51	20.67 $\pm$ 9.77	2.52 $\pm$ 0.64	2.87 $\pm$ 1.67	1.90 $\pm$ 1.02	66.97 $\pm$ 28.14	
	Summer 18	1.19 $\pm$ 0.83	85.07 $\pm$ 17.30	31.85 $\pm$ 9.06	55.74 $\pm$ 11.76	38.26 $\pm$ 8.28	23.40 $\pm$ 7.79	16.37 $\pm$ 6.68	34.60 $\pm$ 10.62	15. $\pm$ 3.72	530.56 $\pm$ 369.19	
Red	Summer 17	0.51 $\pm$ 0.25	28.72 $\pm$ 5.08	14.91 $\pm$ 0.43	19.75 $\pm$ 3.85	19.14 $\pm$ 3.42	8.05 $\pm$ 2.64	7.27 $\pm$ 2.10	15.62 $\pm$ 03.51	10.72 $\pm$ 2.67	447.77 $\pm$ 102.24	
	Autumn 17	0.28 $\pm$ 0.15	3.19 $\pm$ 0.53	4.86 $\pm$ 0.68	1.27 $\pm$ 0.31	1.66 $\pm$ 0.17	19.76 $\pm$ 3.60	1.79 $\pm$ 0.14	0.65 $\pm$ 0.12	2.26 $\pm$ 0.51	59.11 $\pm$ 11.13	
	Winter 17	0.47 $\pm$ 0.05	7.40 $\pm$ 0.53	6.68 $\pm$ 1.11	3.39 $\pm$ 0.24	4.84 $\pm$ 0.68	20.52 $\pm$ 1.63	2.94 $\pm$ 0.77	1.62 $\pm$ 0.21	1.09 $\pm$ 0.26	78.79 $\pm$ 4.02	
	Spring 17	0.23 $\pm$ 0.09	48.88 $\pm$ 27.62	17.42 $\pm$ 0.23	31.09 $\pm$ 17.58	15.40 $\pm$ 3.28	27.54 $\pm$ 8.80	2.02 $\pm$ 0.70	4.95 $\pm$ 2.16	3.98 $\pm$ 2.76	214.41 $\pm$ 169.74	
	Summer 18	0.25 $\pm$ 0.17	21.80 $\pm$ 9.95	14.30 $\pm$ 6.26	12.91 $\pm$ 5.65	12.71 $\pm$ 5.64	7.48 $\pm$ 3.97	2.99 $\pm$ 1.52	11.01 $\pm$ 12.40	9.23 $\pm$ 4.83	210.41 $\pm$ 103.84	

**Table 4** (continued)

Brown	Summer 17	2.91 ± 0.27	50.02 ± 8.39	38.33 ± 6.66	31.79 ± 7.18	24.43 ± 5.29	71.81 ± 14.70	8.19 ± 2.19	27.24 ± 94.46	24.50 ± 3.46	483.86 ± 91.83
	Autumn 17	0.49 ± 0.26	6.32 ± 2.45	9.05 ± 4.33	1.81 ± 0.62	2.15 ± 0.91	27.80 ± 7.35	2.17 ± 0.77	1.72 ± 0.77	2.37 ± 0.56	91.21 ± 35.05
	Winter 17	0.79 ± 0.23	10.68 ± 2.96	8.84 ± 2.83	4.57 ± 1.32	6.69 ± 0.91	19.32 ± 11.76	2.92 ± 0.37	1.12 ± 0.19	1.31 ± 0.44	98.88 ± 18.68
	Spring 17	0.23 ± 0.34	4.85 ± 1.20	14.46 ± 9.02	1.80 ± 0.07	12.65 ± 3.59	9.64 ± 1.631.63	1.37 ± 0.26	2.19 ± 1.22	5.00 ± 3.71	437.75 ± 598.07
G11	Summer 18	0.93 ± 0.00	171.76 ± 3.21	67.59 ± 1.90	105.89 ± 8.41	96.03 ± 3.77	47.88 ± 17.05	28.76 ± 5.76	78.42 ± 4.22	37.18 ± 1.58	1,648.28 ± 67.31
	Summer 17	1.06 ± 0.44	14.78 ± 6.68	12.89 ± 5.81	8.81 ± 4.10	7.66 ± 3.62	36.59 ± 8.76	3.59 ± 1.33	8.12 ± 0.45	6.18 ± 2.14	165.80 ± 76.01
	Autumn 17	0.57 ± 0.17	8.84 ± 0.67	12.58 ± 2.46	2.43 ± 0.14	3.34 ± 0.14	46.03 ± 3.88	3.29 ± 0.66	2.73 ± 0.18	2.47 ± 0.86	168.73 ± 85.88
	Winter 17	0.55 ± 0.16	24.70 ± 9.67	15.56 ± 6.47	21.69 ± 8.30	21.21 ± 7.23	12.58 ± 7.91	3.97 ± 1.12	12.15 ± 4.63	11.26 ± 3.30	257.79 ± 87.94
Spring 17		2.01 ± 0.57	147.22 ± 23.86	41.02 ± 12.23	109.51 ± 20.68	51.36 ± 10.42	43.25 ± 9.74	18.58 ± 2.78	47.8 ± 8.11	23.47 ± 1.42	817.27 ± 132.91
	Summer 18	0.81 ± 0.13	74.63 ± 0.74	36.26 ± 0.22	48.01 ± 0.39	43.73 ± 0.06	35.47 ± 2.71	13.33 ± 2.03	39.65 ± 0.83	18.10 ± 0.57	585.55 ± 364.36

*alvarezii* analyzed had enough mineral content for recommended daily intake (Ottén et al. 2016), but the G11 strain had highest values in Fe, Ca, and Mg.

The highest protein levels were observed in the brown and red strains. These variations of protein concentration among *K. alvarezii* strains may be related to differences in physiological and chemical profiles of each strain, as was also observed in this study in the carbohydrate levels and antioxidant properties. According to Kumar et al. (2014), *K. alvarezii* may be used as an inexpensive source of protein, incorporating into several value-added food products or in feed.

The finding that carbohydrate content represented the major nutritional constituent of *K. alvarezii* with up  $231.79 \pm 23.03 \text{ mg g}^{-1} \text{ DW}$  supports the use of this seaweed as a source of carbohydrates and dietary fiber. In addition, Masarin et al. (2016) reported greater carbohydrate values for the same *K. alvarezii* cultivated on the São Paulo coast with mean total of  $534 \text{ mg g}^{-1} \text{ DW}$ . The carbohydrate content reported for *K. alvarezii* farmed in India was similar to values observed in the present study, ranging from  $230 \pm 16.40$  to  $275 \text{ mg g}^{-1} \text{ DW}$  (Fayaz et al. 2005; Kumar et al. 2015). Other carbohydrate content reports for species of *Kappaphycus* were published for seaweeds farmed in Indonesia with  $52.4 \pm 16.40 \text{ mg g}^{-1} \text{ DW}$  (Adharini et al. 2020), in Malaysia with 625 to 666 mg of carbohydrate  $\text{g}^{-1} \text{ DW}$  (Ahmad et al. 2012), and in the Philippines with  $679 \text{ mg g}^{-1} \text{ DW}$  (Alcantara and Lazaro-Llanos 2020). In addition to applying *K. alvarezii* as a carrageenan source used in the food and pharmaceutical industries as a gelling and thickening agent (Hurtado et al. 2015), the high carbohydrate content of this species can be explored as a source of dietary fiber (Adharini et al. 2020). Additionally, seaweed supplementation for animal food based on the prebiotic action of their complex carbohydrates has increased in recent decades, reducing the use of antibiotics (Ariano et al. 2021). Seaweed-based biorefinery to generate biofuels, such as bioethanol, could be another high-value application (Masarin et al. 2016).

Although the carbohydrate contents differed significantly among strains, with the highest levels occurring in the red and brown strains of *K. alvarezii*, such differences were small, and no strain was specifically identified as the best carbohydrate source. Similarly, Masarin et al. (2016) observed a significant difference of carbohydrate content among strains of *K. alvarezii*, but such variations were minimal.

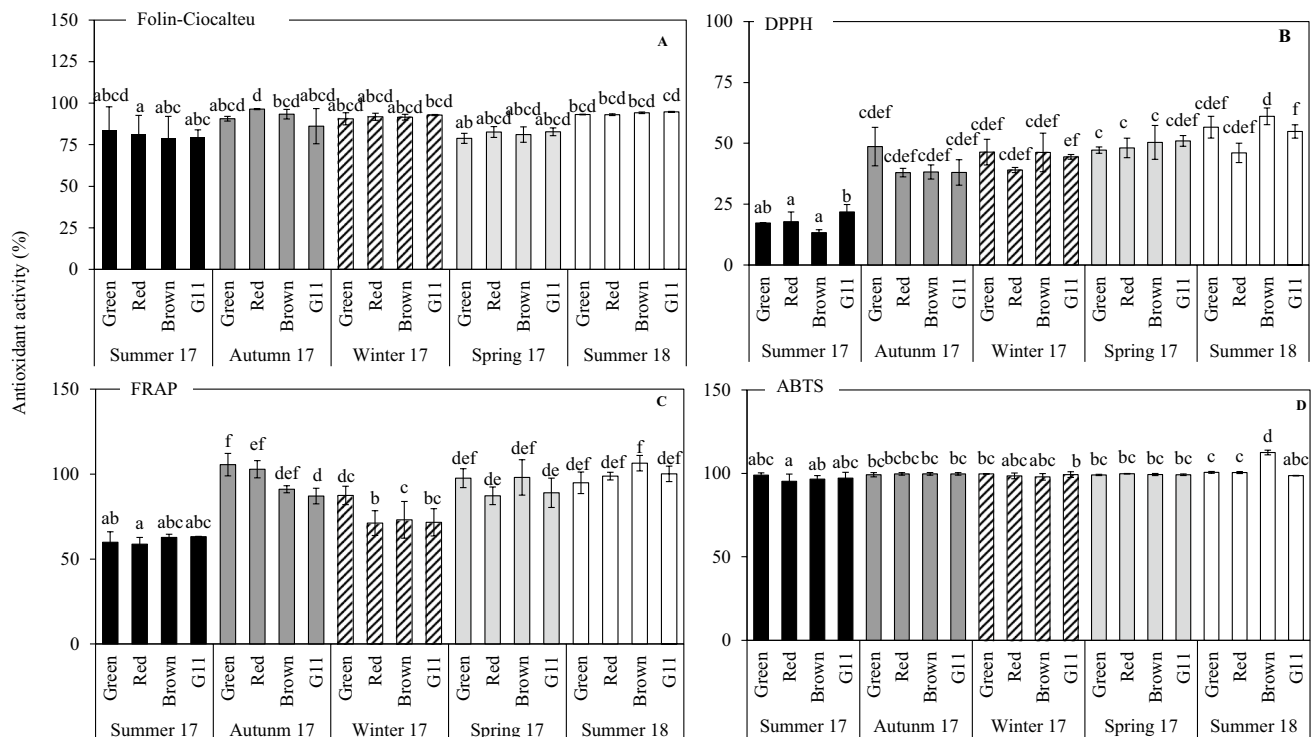
The fluctuation of carbohydrate content occurred seasonally, with the highest values in summer 2018. This highest carbohydrate content of *K. alvarezii* in summer 2018 could indicate a period of greater growth of this seaweed, as a consequence of favorable environmental conditions of spring 2017 and summer 2018 for its development. While an understanding of the nutritional content of

**Table 5** Total phenolic content (mg GAE g<sup>-1</sup> DW) of four strains of *Kappaphycus alvarezii* in all seasons. Values are mean ( $n=5$ )  $\pm$  *sd*

Strain	Summer 17	Autumn 17	Winter 17	Spring 17	Summer 18
Green	53.31 $\pm$ 17.76 <sup>a</sup>	73.16 $\pm$ 2.28 <sup>bc</sup>	52.92 $\pm$ 4.26 <sup>a</sup>	75.87 $\pm$ 6.82 <sup>bc</sup>	99.09 $\pm$ 2.17 <sup>de</sup>
Red	40.57 $\pm$ 17.47 <sup>a</sup>	132.40 $\pm$ 0.88 <sup>g</sup>	55.96 $\pm$ 2.55 <sup>a</sup>	105.19 $\pm$ 9.13 <sup>ef</sup>	119.63 $\pm$ 1.30 <sup>fg</sup>
Brown	44.94 $\pm$ 1.26 <sup>a</sup>	100.43 $\pm$ 6.01 <sup>de</sup>	53.70 $\pm$ 2.02 <sup>a</sup>	108.16 $\pm$ 13.94 <sup>ef</sup>	91.59 $\pm$ 0.89 <sup>de</sup>
G11	44.81 $\pm$ 4.64 <sup>a</sup>	99.56 $\pm$ 25.69 <sup>de</sup>	64.03 $\pm$ 0.47 <sup>ab</sup>	102.81 $\pm$ 6.40 <sup>def</sup>	84.44 $\pm$ 0.47 <sup>cd</sup>
Mean	46.26 $\pm$ 12.52 <sup>A</sup>	101.39 $\pm$ 24.73 <sup>B</sup>	56.65 $\pm$ 5.15 <sup>A</sup>	98.01 $\pm$ 15.48 <sup>B</sup>	98.68 $\pm$ 13.63 <sup>B</sup>

Superscript letters derived from post hoc Newman–Keuls after ANOVA test ( $P < 0.05$ ) indicate significant differences among seasons

\*Significance ( $F = 16.51$ ,  $p < 0.05$ )



**Fig. 7** Antioxidant activity of four strains of *Kappaphycus alvarezii* by different antioxidant assays in the studied seasons. Letters indicate the differences between strains and seasons as post hoc Newman–

Keuls after factorial ANOVA ( $p < 0.05$ ). (A) Folin-Ciocalteu assay, (B) DPPH assay, (C) FRAP assay and, (D) ABTS assay

seaweeds according to strains and seasons is fundamental, it is also important to consider the productivity rates of cultivation for better commercial application. According to Hayashi et al. (2007), the G11 strain of *K. alvarezii* had the lowest growth rates; however, as a compensatory factor, it had a carrageenan yield higher than the other strains cultivated.

Antioxidant assays to evaluate activity might not measure the real antioxidant properties, but the utilization of different and complementary assays may provide excellent information to support the better exploitation of antioxidant properties of *K. alvarezii* (Gereniu et al. 2017; Araújo et al. 2020). TPC of *K. alvarezii* farmed on the São Paulo coast was

greater than TPC reported for the same species cultivated in Cambodia ( $28.4 \pm 1.1$  mg GAE g<sup>-1</sup> DW) (Chew et al. 2008) and Malaysia ( $7.51 \pm 0.16$  to  $19.17 \pm 0.04$  mg GAE g<sup>-1</sup> DW) (Farah Diyana et al. 2015; Farah Nurshahida et al. 2020). Rates of DPPH radical-scavenging activity constituted the lowest antioxidant potential among assays analyzed between  $13.29 \pm 1.20$  and  $61.07 \pm 3.43\%$ . However, these values are still within of interval of 7.5 to 82% reported for *K. alvarezii* (Fayaz et al. 2005; Kumar et al. 2008; Farah Diyana et al. 2015; Gereniu et al. 2017; Farah Nurshahida et al. 2020).

Antioxidant potential of different strains of *K. alvarezii* varied according to the season. As such, no strain could be considered the best antioxidant source the whole year round.

**Table 6** Composite index of nutritional and antioxidant potential of *Kappaphycus alvarezii* farmed on the Brazilian coast

Season	Strain	Carbohydrate	Total minerals	Amino acid	Total AOX	Mean
Summer 17	Green	41.63	97.53	75.63	17.21	58.00
	Red	66.76	65.68	3.59	16.99	38.25
	Brown	38.37	88.77	6.00	14.21	36.84
	G11	41.92	72.84	49.58	14.25	44.65
	<b>Mean</b>	<b>47.17</b>	<b>81.21</b>	<b>33.70</b>	<b>15.66</b>	<b>44.44</b>
<b>Green &gt; G11 &gt; Red &gt; Brown</b>						
Autumn 17	Green	66.11	100.00	27.17	32.63	56.48
	Red	65.44	64.58	5.53	52.01	46.89
	Brown	93.49	54.71	15.64	45.38	52.30
	G11	60.42	66.37	32.19	48.88	51.96
	<b>Mean</b>	<b>71.36</b>	<b>71.42</b>	<b>20.13</b>	<b>44.73</b>	<b>51.91</b>
<b>Green &gt; Brown &gt; G11 &gt; Red</b>						
Winter 17	Green	79.29	67.93	29.36	25.49	50.52
	Red	85.83	85.13	10.24	24.54	51.43
	Brown	85.76	72.58	4.06	23.06	46.37
	G11	99.40	86.13	12.77	28.35	56.66
	<b>Mean</b>	<b>87.57</b>	<b>77.94</b>	<b>14.11</b>	<b>25.36</b>	<b>51.24</b>
<b>G11 &gt; Red &gt; Green &gt; Brown</b>						
Spring 17	Green	80.11	80.64	10.06	78.52	62.33
	Red	90.42	73.53	19.90	87.79	67.91
	Brown	94.90	72.96	13.04	100.00	70.22
	G11	74.20	65.35	100.00	67.80	76.84
	<b>Mean</b>	<b>84.91</b>	<b>73.12</b>	<b>35.75</b>	<b>83.53</b>	<b>69.33</b>
<b>G11 &gt; Brown &gt; Red &gt; Green</b>						
Summer 18	Green	78.80	70.46	6.59	63.15	54.75
	Red	100.00	85.58	4.78	74.21	66.14
	Brown	90.63	69.54	26.56	61.53	62.06
	G11	86.40	61.28	35.53	51.61	58.70
	<b>Mean</b>	<b>88.96</b>	<b>71.72</b>	<b>18.36</b>	<b>62.62</b>	<b>60.41</b>
<b>Red &gt; Brown &gt; G11 &gt; Green</b>						
<b>Spring 17 &gt; Summer 18 &gt; Autumn 17 &gt; Winter 17 &gt; Summer 17</b>						

Values in bold indicate composite index results for each season and final result

However, considering the summer of 2017, the green strain had the best antioxidant potential, corroborating the finding reported by Araújo et al. (2020). In autumn 2017 and summer 2018, the red strain was the best for antioxidant activity, but in winter 2017, it was the G11 strain, and in spring 2017, it was the brown strain.

The inverse correlation between antioxidant potential of *K. alvarezii* and water temperature suggests that the cool seasons are the best periods for utilization of seaweed as an antioxidant source. This recommendation may be a feasible solution of adding value during a time of low productivity since the productivity and growth rates of *K. alvarezii* are lower in times of low seawater temperature (Hayashi et al. 2007; Solorzano-Chavez et al. 2019).

The CAPC Index compiles, weights, and orders the chemical and antioxidant descriptors of *K. alvarezii*. Considering the strains by season, green strains had the best chemical

and antioxidant profiles in summer and autumn 2017, while G11 had the best chemical and antioxidant profiles in winter and spring 2017, and the red strain was the best option in summer 2018. These results indicate differences between *K. alvarezii* strain physiology, variation of pigments content, and chemical composition of this lineage, influencing physiology, growth, and biological activity of this lineage (Hayashi et al., 2007; Araújo et al. 2014, 2020). Considering season, the spring of 2017 was the best season for chemical and antioxidant profiles of *K. alvarezii*, as already discussed above.

Therefore, *K. alvarezii* produced along the São Paulo coast has excellent chemical and antioxidant properties with potential applications as a nutritional supplement, functional food and feed, and biofertilizer. These chemical and antioxidant characteristics vary according to strains and season with lower temperature as the best period for the application of seaweed as a source of antioxidants and nutrition.



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## Declarations

**Conflict of interest** The authors declare no competing interests.

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