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Nutrient composition and content of various biological active compounds of three South African-based commercial seaweed biostimulants

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Abstract Positive results reported in commercial agriculture following the application of seaweed (kelp) products vary from root growth stimulation to increased disease resistance. The described impact of seaweed applications is mostly reliant on the specific species of seaweed used, the prevalent environmental growing conditions, together with the extraction and formulation protocol implemented in the production of the commercial extracts. These possible variables alone or in combination may lead to inconsistencies in efficacy between different commercial seaweed products, especially under field conditions. In this paper, we quantify selected active components in three different, but apparently similar, commercial seaweed products manufactured from *Ecklonia maxima* which could impact on biological activity, following application as evaluated for mung bean root growth. Significantly higher P and N concentrations were found both in Afrikelp® and Basfoliar® Kelp compared to Kelpak®. These elevated mineral nutrient concentrations indicate possible enrichment of these products, as they exceed the natural nutrient element concentrations in the freshly milled *E. maxima* prior to formulation. Kelpak® showed higher concentrations of Ca, Mg and K compared to Afrikelp® and Basfoliar® Kelp, but lower levels were reported to occur naturally in freshly minced seaweed from *E. maxima*. Concentrations of mannitol, uronic acid and neutral sugars together with alginic acid content were significantly higher in Kelpak® than in Afrikelp® and Basfoliar® Kelp. Similarly, mung bean root growth stimulation was significantly higher in Kelpak® than in Afrikelp® and Basfoliar® Kelp. This study showed that commercial seaweed products, manufactured from the same seaweed source such as *E. maxima*, and thus generally marketed as equivalent products, may vary significantly in product composition and thus in efficacy to induce specific plant responses following application, when manufactured by different companies. Distinct differences in harvesting and manufacturing protocols place an obligation on the end user to ensure that the composition of the product of choice is well aligned with the specific plant response required.

Keywords Alginic acid · *Ecklonia maxima* · Kelp · Mannitol · Nutrients · Uronic acid

Introduction

Agricultural biostimulants are natural products that may enhance crop productivity, especially when applied to crops resorting under conditions of stress. Novel biological modes of action employed by these compounds are distinctly different than those associated with conventional fertilizers (Sharma et al. 2014). Biostimulants are therefore mostly used complementary to strategies designed specifically for crop nutrition and crop protection. Seaweed species act as an important source for the production of biostimulants. At present, seaweed is considered the single most researched and widely used type of biostimulant (Du Jardin 2012), with the global seaweed processing industries utilizing an estimated biomass of 10 to 12 million tonnes annually (Nayar and Bott 2014).

Positive results on plant-growth-enhancing properties together with increased disease resistance and tolerance to climatic stresses such as cold or drought following application of seaweed extracts have been reported (Featonby-Smith and Van Staden 1988; Colavita et al. 2011; Briceño-Domínguez

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et al. 2014; Arioli et al. 2015). Chemical analysis of brown macroalgae has shown these aquatic species to be naturally rich in the macro elements Ca, Mg, K, Na, P and S, as well as in some trace elements, including Fe (Verkleij 1992; Sharma et al. 2014). In addition to the presence of mineral elements, the promoting effects on root growth stimulation, improved leaf development, as well as enhanced flowering and fruit set and biomass have been ascribed to the many types of biologically active molecules of plant growth regulators known to be present in seaweed. These metabolites comprise auxins, cytokinins, ethylene and gibberellins and more recently were reported to also include considerable amounts of polyamines (Papenfus et al. 2012), abscisic acid and brassinosteroids (Stirk and van Staden 1997; Stirk et al. 2014). Levels of auxins and cytokinins have been identified in fresh kelp similar to those of turgid leaves of higher plants (Verkleij 1992), but varying in levels according to species and growing conditions (Bolton and Anderson 1990). Another range of compounds reported in seaweed extracts comprise several osmo-protectants such as the quaternary ammonium compounds betaine and proline, together with the common storage sugar-alcohol, mannitol (Craigie 2011; Calvo et al. 2014). Where betaines and proline provide a buffer against stress conditions, mannitol plays an important role in osmo-regulation, while offering stress mitigation through the modes of coenzyme regulation, free-radical scavenging and enhanced resistance to pathogens (Stoop et al. 1996; Bohnert and Jensen 1996; Prabhavathi and Rajam 2007; Vera et al. 2011). Additional components of major biological interest, especially for their reported stimulation of nutrient uptake, translocation and ability to stimulate root growth (Craigie 2011), directly or indirectly when in association with microbes, are alginates and some diverse polysaccharides (Khan et al. 2012; González et al. 2013). Sodium alginate is a gum and salt of alginic acid that is extracted from the cell walls of brown algae, with the physiological and rheological properties greatly influenced by the uronic acid composition (Haug and Larsen 1962). When seaweed extract was applied to growth substrates, it was reported to enhance soil conditions, with the alginic acid action targeted at stimulating the bacterial decomposition of organic material (Zodape 2001). Soluble alginates from seaweed are known to act as an aggregation facilitator between soil particles, thus resulting in increased nutrient uptake (Manley 1981; Verkleij 1992).

Commercial liquid seaweed extracts are complex as several sources of variation all contribute to unique product formulations. Firstly, the range of seaweed species used (e.g. *Ascophyllum nodosum*, *Durvillaea potatorum* and *Ecklonia maxima*) as well as the unique origin of the seaweed may be the basis for distinct, inherent differences (Arioli et al. 2015). In South Africa, several products are produced from the local seaweed species *E. maxima*, whereas other imported products such as the French-based Göemar, produced from *A. nodosum*, are also available (Craigie 2011).

Secondly, the various extraction processes which include alkaline and acid hydrolysis or pressure differential cell bursting or fermentation will impact on the extract stability properties. Where the process of cold cell bursting is achieved through rapid changes in cell pressure to rapture the cells, most commercial extracts are produced by alkaline hydrolysis using sodium hydroxide, sodium carbonate or potassium carbonate. The physiochemical properties and plant-growth-promoting activity may further be affected by the pH and temperature at which the chemical extractions are performed. Briceño-Domínguez et al. (2014) reported extract viscosity to increase with an increasing pH and temperature, up to maximum at pH 10 and 80 °C.

A third factor that contributes to variation between seaweed-based biostimulants exists within the formulation protocol. In order to release cellular content from crude seaweed extracts, nutrient additions of chelated trace elements as well as the macro elements N, P and K to the raw seaweed extract are often used (Verkleij 1992; Craigie 2011). In a comparison of the chemical composition of the two commercial seaweed products manufactured from *A. nodosum* (Maxicrop™ and Algifert™), significant differences in Fe, Cu and B concentrations were revealed (Craigie 2011). A fourth source of variation between manufacturers in the commercial formulation of the various seaweed products sold for agricultural use may occur when preservatives are added to the liquid extracts to prevent microbial contamination (Verkleij 1992). This will, in addition to the abovementioned factors, also result in a unique product formulation which is distinctly different from the raw seaweed composition used as a base extract.

The aims of the study were (i) to quantify the nutrient composition and content of various biologically active compounds within three South African-produced commercial seaweed products originating from *E. maxima* and (ii) to evaluate the application thereof as a root stimulant on mung beans to assess whether these products can be considered and used as equivalent biostimulants, as applicable within an agricultural context.

Materials and methods

Seaweed biostimulants

Various 25 L containers of Kelpak® (Kelp Products (Pty) Ltd, Simon's Town, South Africa) and Afrikelp® (Afrikelp (Pty) Ltd, Milnerton, South Africa), representing different production batches, as purchased in 2011 and 2013, respectively, from suppliers in the Western Cape, South Africa, were used. Kelpak® purchased in 2011 refers to batches 1310.11, 501.11 and 22.10.11, with the 2013 purchase referring to batches 06.11.12, 21.11.12 and 19.01.13, while the 2011 Afrikelp®

purchase included batches 100501, 101001 and 101101, with the 2013 purchase being represented by batches 120301F, 110801F and 1105011. Smaller 1 L to 10 L containers of Basfoliar® Kelp (COMPO GmbH, Chile, produced by Afrikelp Pty Ltd) were imported in 2013 from Chile, Dominiqua, Argentinia, Mexico and Spain, respectively. All three products are manufactured from *E. maxima*, harvested on the South African coastline. Afrikelp® (http://www.afrikelp.com/?m=3) uses a cold micronization process (CMP) followed by filtering to 30 μm, whereas Kelpak® (www.kelpak.com) uses the cold cell bursting technique process (Stirk and Van Staden 1997) followed by centrifugation (AF Lourens, personal communication). Samples of Kelpak®, Afrikelp® and Basfoliar® Kelp were collected in clean 500 mL plastic bottles for chemical analysis, according to a randomized design of six replications.

Additionally, more than 300 kg fresh *E. maxima* seaweed of individual commercial sizes of approximately 2 m in length was harvested in October 2013, both from Kommetjie (34° 5′ S, 19° 3′ E) for Kelpak® production (Kelpak (Pty) Ltd) as well as from the Gansbaai (34° 2′ S, 18° 3′ E) harvesting site for Afrikelp® and Basfoliar® Kelp production. Sites are geographically located approximately 130 km apart, on the southern west coast of South Africa. The seaweed from the respective sites was separated into stipes and fronds and minced with an industrial meat mincer (4 mm particle size), whereafter it was mixed with 1 % peracetic acid as an antimicrobial agent and preservative, before being passed through an emulsifier to reduce particle sizes to 1 mm. The samples were then transferred for alginate content determination by Aquagri Processing Private (Pty) Ltd (New Delhi, India) and Bemlab Pty Ltd (Strand, South Africa) for mineral analysis.

Mineral analyses

Mineral analyses were performed by Bemlab (Pty) Ltd (Strand, South Africa). Nitrogen levels were determined using a nitrogen analyser (Method AOAC 984.13), while the phosphorous (Method AOAC 965.17) and a full mineral (Method AOAC 968.08) content range were determined using ICP-OES. Free phosphate concentration was determined by the Department of Horticultural Science at Stellenbosch University, according to the method of McGinley (2011).

Mannitol, uronic acid and neutral sugar content

Mannitol, uronic acid and neutral sugar content were determined according to the protocol of McGinley (2011). Mannitol was analysed with an Agilent HPLC (1100 Series; Germany), equipped with a refractive index detector (Model G1352A; Agilent), on a Transgenomic ION-300 column (300×7.8 mm; ICSep ICE-99-9850; Transgenomic, USA) linked to a guard column (ICSep ICE-GC-801; Transgenomic). The column temperature was maintained at 35 °C, with an injection volume of 20 μL, and a set flow rate of 0.4 mL.min^{-1}, using 5 mM H_2SO_4 as the eluent. External standards (0–500 mg L^{-1}) of D-mannitol (Merck; SAAR4125500EM) were used as external standard. Kelp samples were prepared for analysis by making fivefold diluted aliquots taken from the thoroughly mixed commercial products, whereafter the diluted samples were centrifuged at 14,000×*g* for 10 min at 20 °C, and the supernatant filtered through a 0.45-μm syringe filter prior to injection. Uronic acid and the neutral sugar content was determined according to methods of Filisetti-Cozzi and Carpita (1991) and Tabarsa et al. (2013), respectively.

Alginic acid

Alginic acid was determined by the Centre for Bioprocess Engineering, University of Cape Town, according to a modified assay of Dubois et al. (1956). For the assay, 1-mL kelp samples were added, in triplicate, to 2-mL Eppendorf tubes. A calibration curve was constructed by pipetting a range of 1-mL aliquots of sodium alginate at 0.25, 0.5, 1 and 1.5 g L^{-1} to 2-mL Eppendorf tubes. To these samples, 1 mL 1 M HCl was added to precipitate the alginate. Tubes were then vortexed and centrifuged at 13,000×*g* for 10 min, whereafter the supernatant was discarded. To the solid alginate pellet, 2 mL of 0.5 M HCl was added to remove any contaminating sugars and colourants. The solid pellet was re-suspended by vortexing, and the tubes centrifuged for another 10 min at 13,000×*g*, whereafter the supernatant was discarded. To the solid pellet, 3 % Na_2CO_3 solution was added to a volume of 1 mL, in order to solubilize the alginate. The pellet was then re-suspended by vortexing, whereafter the tube was placed in a 50 °C water bath for 20 min. The tubes were then centrifuged at 4000×*g* for 1 min to remove any remaining contaminating solids, whereafter the supernatant was retained for analysis. In order to test for alginates, a sulphuric acid-phenol mixture which consisted of 50 μL 80 % aqueous phenol added to 5 mL of concentrated sulphuric acid was freshly prepared. In a 2-mL Eppendorf tube, 0.47 mL of prepared kelp sample was combined with 1.43 mL of the sulphuric acid-phenol solution and vortexed, with the absorption determined directly afterwards at 480 nm.

Amino acids

For Kelpak® and Afrikelp®, respectively, the three product batches were pooled per year of purchase in similar ratios to form a composite sample per product for a particular production year (2011 and 2013). For the 2013 purchase of Basfoliar® Kelp, the products which originated from Chile, Argentina and the Dominican Republic were pooled in an equal ratio, whereas the products produced in Mexico, Chile

and Spain were similarly pooled to form a second composite sample of Basfoliar® Kelp. Sufficient seaweed liquid volumes per composite sample were dried using a rotary evaporator at 22 °C to obtain final solid weights for each sample of approximately 2 g dry weight (DW) per product. The solids were then analysed at the Center of Analytical Facilities (CAF) at Stellenbosch University for amino acid contents, according to the protocol of Grobbelaar et al. (2014). Amino acids analyses were performed on the composite samples of the three biostimulant products.

Mung bean bioassay

Biological auxin-like response as rooting activity to the respective seaweed biostimulants was evaluated with a mung bean bioassay performed according to Crouch and Van Staden (1991) at the Research Centre for Plant Growth and Development, University Kwazulu-Natal (Pietermaritzburg, South Africa). Seeds of the mung bean (*Vigno mungo* L.) were planted in vermiculite trays and germinated at 26 °C. After 9 days, 20 uniform hypocotyl cuttings at 120 mm, with 2 primary leaves and without cotyledons, were transferred according to a randomized block design to the 4 treatment vials at 5 cuttings per vial. Vials were filled with 20 mL of the respective treatment solutions and cuttings were soaked for 8 h at 24 °C under lighting conditions of 140 μmol photons m^{-2} s^{-1}. Following this pulse treatment, the bases of the cuttings were removed, whereafter the cuttings were thoroughly rinsed with tap water before being transferred to clean vials containing distilled water only. After 8 days of incubation, the number of roots per hypocotyl was recorded.

A dose response curve for rooting ability was performed using a concentration range of 0, 0.02, 0.5, 2.0, 5, 10, 20, 100 and 200 mg L^{-1} indole-3-butyric acid (IBA). Kelpak®, Afrikelp® and Basfoliar® Kelp samples were diluted in a concentration range of 1, 2, 5, 10, 20 and 50 %, and the rooting ability of each solution was determined. All treatments and the dose response curve were performed at the same time to ensure equal exposure to similar environmental growth conditions for all experimental units. The average number of roots per vial was used as replicates for LSD and standard error calculations.

Statistical analyses

Statistical analyses were performed using the GLM procedure with SAS 9.3 (SAS Institute Inc, USA). Where appropriate, data were analysed for statistical differences by analysis of variance (ANOVA), with mean separation using least square means and least significant differences (LSD). The standard error of the mean (SE values) was also calculated where appropriate. Significance was determined at 5 % ($P<0.05$). For amino acid composition analysis, statistical analysis of the data was not possible due to insufficient replication and thus trends were discussed. The pooled nature of the composite sample used for the alginate and mineral analyses of the crude *E. maxima* extracts rendered it unsuitable for statistical analysis of data, and again, trends were discussed.

Results

Mineral nutrient and alginate content

Results from the mineral composition and alginate content of raw *E. maxima* fronds and stipes as harvested in Gansbaai and Kommetjie in October 2013 showed a tendency of higher levels of N, P, Ca and Mg, as well as alginate percentages in the fronds compared to the stipes (Table 1). K concentrations exhibited a reverse trend, with higher concentrations in the stipes than in the fronds (Table 1).

N, P and K concentrations tended to be higher in the seaweed which was harvested from Kommetjie, whereas the alginate percentage appeared to occur at elevated levels in the material collected from Gansbaai. Ca and Mg concentrations were recorded to occur in comparable levels between the two respective harvesting sites (Table 1).

The Kelpak® formulation showed significantly higher levels of K, Ca, Mg and Na concentrations compared to the Afrikelp® and Basfoliar® Kelp formulations, which were similar (Table 2). N and P concentrations were significantly

Table 1 Mineral composition and alginate content (g L^{-1}) of raw *Ecklonia maxima* extracts as harvested in October 2013 at Gansbaai and in Kommetjie. Kelp harvested for Afrikelp® and Basfoliar® Kelp production are obtained from Gansbaai whereas kelp used in the production of Kelpak® is collected from Kommetjie

Ecklonia maxima Source	N %	P mg kg^{-1}	K mg kg^{-1}	Ca mg kg^{-1}	Mg mg kg^{-1}	Alginate g L^{-1}
Gansbaai fronds	0.18	101	66	17	6	4.18
Gansbaai stipes	0.10	10	102	11	2	2.38
Kommetjie fronds	0.21	4	111	14	7	2.61
Kommetjie stipes	0.16	5	188	14	3	1.68

Table 2 Mean mineral composition of three liquid seaweed (kelp; *Ecklonia maxima*) product formulations Afrikelp®, Basfoliar® Kelp and Kelpak® (n=6) as purchased in 2011 and 2013, respectively. Data is expressed as mg kg^{-1} fresh weight

Macro elements	N	P	K	Ca	Mg	Na
	%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Afrikelp®	0.26 a±0.04	3761.0 a±660.72	403.0 b±105.23	29.2 b±10.30	41.0 b±6.45	359.2 b±71.00
Basfoliar®	0.28 a±0.05	3960.0 a±659.64	581.0 b±273.61	21.2 b±10.40	37.2 b±16.67	317.7 b±19.96
Kelpak®	0.09 b±0.01	90.7 b±34.48	7163.3 a±570.13	190.4 a±50.90	337.2 a±33.67	1623.7 a±84.46
P	0.005	0.0002	<0.0001	0.0018	<0.0001	<0.0001
Micro elements	Mn	Fe	Cu	Zn	B	
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	
Afrikelp®	7.5±2.33 ns	16.8±5.08 ns	4.8±2.47 ns	5.2±2.15 ns	16.0±6.11 ns	
Basfoliar®	2.0±0.86	9.0±1.63	0.17±0.17	1.0±0.37	2.7±0.49	
Kelpak®	17.3±12.32	40.7±20.79	13.5±10.79	17.0±11.86	33.0±14.14	
P	0.3441	0.2039	0.3108	0.3056	0.0849	

Data are presented as mean±standard error of the mean; similar letters within a column are not significantly different at the 5 % level

ns not significant

higher in Afrikelp® and Basfoliar® Kelp compared to Kelpak® and substantially higher than naturally occurring levels. The micro elements Mn, Cu, Fe, Zn and B concentrations were not significantly different between products (Table 2). These low micronutrient levels are unlikely to contribute significantly towards plant nutrition under normal growing conditions.

Mannitol, uronic acid, neutral sugar, free phosphate, alginic acid content and amino acid composition

Mannitol, uronic acids and neutral sugar levels in Kelpak® were found to be significantly higher compared to those of Afrikelp® and Basfoliar® Kelp, which were similar (Table 3). Significantly higher free P concentration was found in Afrikelp® and Basfoliar® Kelp compared to Kelpak® (Table 3). Kelpak® also had significantly higher alginate levels than Afrikelp® and Basfoliar® Kelp, which again did not differ significantly (Table 3).

For all analysed amino acids, the concentrations in Kelpak® were higher than those recorded in Afrikelp® and Basfoliar® Kelp (Table 4). The total amino acid concentration was thus considerably higher in Kelpak® than in Afrikelp® or Basfoliar® Kelp (Table 4). Histidine was below the limit of detection in the Afrikelp® or Basfoliar® Kelp samples analysed, whereas cysteine was detected in only one of the Basfoliar® Kelp samples.

Mung bean bioassay

The mung bioassay which was used to evaluate the auxin-like activity of the seaweed products showed that Kelpak® at the lower concentration range of 1 and 2 % stimulated root production more efficiently than was recorded for Basfoliar® Kelp and Afrikelp®. Kelpak® at 5 % dilution produced more roots on the hypocotyls than Afrikelp® at 50 % but did not differ significantly from Basfoliar® Kelp. Afrikelp® and Basfoliar® Kelp consistently showed similar rooting activity at the different concentrations evaluated. Rooting stimulation for all the products reached an optimum at 20 % dilution, whereafter at 50 % dilution a decline in rooting stimulation for all evaluated products was observed (Table 5).

Discussion

With the expansion of commercially available seaweed-based products on offer, significant differences on the biological efficacy associated with competitor products have been

Table 3 Analyses of the seaweed (kelp *Ecklonia maxima*) products Afrikelp®, Basfoliar® Kelp and Kelpak® (n=6) for free phosphate, mannitol, uronic acid and neutral sugar levels and alginic acid content

Treatment	Free phosphate (mM)	Mannitol (mg L^{-1})	Uronic acids (g L^{-1})	Neutral sugars (g L^{-1})	Alginate (g L^{-1})
Afrikelp®	128.1 a±24.58	12.8 b±8.17	0.27 b±0.03	0.14 b±0.06	0.1 b±0.03
Basfoliar®	134.6 a±21.79	29.7 b±29.67	0.14 b±0.04	0.06 b±0.02	0.1 b±0.03
Kelpak®	1.7 b±0.38	2261 a±118.60	1.66 a±0.23	1.08 a±0.12	1.5 a±0.19
P	0.0005	<0.0001	<0.0001	<0.0001	<0.0001

Data are presented as average±standard error of the mean; similar letters within a column are not significantly different at the 5 % level

Table 4 Amino acid analysis of composite samples of three commercial seaweed formulations Afrikelp®, Basfoliar® and Kelpak® (n=1) as was purchased during 2011 and 2013, respectively

Biostimulant product	Year/rep	His	Ser	Arg	Gly	Asp	Glu	Thr	Ala	Pro	Cys	Lys	Tyr	Met	Val	ILe	Leu	Phe	Total
mg.100 g^{-1}																			
Kelpak®	2011	4.9	18.5	20.2	23.0	74.8	79.5	19.6	70.2	19.9	0.4	18.3	6.9	3.0	25.0	14.3	23.5	14.7	436.7
Kelpak®	2013	4.6	23.1	17.7	26.1	67.3	78.5	22.9	60.9	21.3	0.3	20.5	9.2	2.3	28.4	16.1	25.4	16.7	441.3
Afrikelp®	2011	0.0	3.0	2.7	5.6	7.1	12.0	3.8	5.3	3.3	0.0	3.4	2.2	5.0	4.6	2.4	4.2	2.8	67.4
Afrikelp®	2013	0.0	2.2	1.4	4.2	8.7	13.4	2.7	3.9	2.3	0.0	1.8	1.1	5.5	4.2	1.5	2.8	1.9	57.6
Basfoliar®	1–3	0.0	1.8	1.2	4.8	6.3	9.5	1.7	3.1	1.4	0.0	1.5	1.1	5.2	2.3	0.8	1.8	1.6	44.1
Basfoliar®	4–6	0.0	1.5	0.7	3.3	7.0	10.1	1.7	2.3	1.3	0.2	1.4	0.6	5.3	1.8	0.8	1.1	0.7	39.8

Amino acid abbreviations are explained from left to right as follows: histidine, serine, arginine, glycine, aspartic acid, glutamic acid, threonine, alanine, proline, cysteine, lysine, tyrosine, methionine, valine, isoleucine, leucine, phenylalanine

experienced. This variability in apparently similar products may negatively impact on the reputation of seaweed as a reliable, consistent and effective biostimulant. Acknowledging the significant variation associated with the efficacy of seaweed products, Basak (2008) appealed for more in-depth knowledge of the biochemical characteristics of different seaweed extracts. Such information would provide a deeper understanding regarding the interaction of the various components which in turn would enable the development of protocols with high efficacy, applicable on different crops, at their various development stages.

Seaweed is a natural product which is not limited to the harvesting area or species of choice as far as product manufacturing is concerned. Variation which is known to occur is associated with the inherent variation in composition between fronds and stipes, seasonal variation in the concentration of certain active components and extraction method employed, to name but a few. Consistency of results when seaweed products are applied under field conditions is further complicated by the target crop and application method. It is therefore of paramount importance to understand the composition of the kelp product of choice so as to ensure the best match in order to achieve the desired results for a specific application requirement.

When mineral content was considered, the significantly higher P and N concentrations reported in Afrikelp® and Basfoliar® Kelp compared to those in Kelpak® may indicate an additional permissible enrichment of these products, all produced from *E. maxima*, as natural levels in the freshly milled seaweed prior to processing were found to be much lower (Tables 1 and 2). Similarly, significantly higher P levels in Afrikelp® and Basfoliar® Kelp products were reported in this study, also as pertaining to free phosphate levels (Tables 1, 2 and 3). The increased root and vegetative growth pledged by manufacturers following the application of the Afrikelp® and Basfoliar® Kelp products may therefore partly be due to generally higher P levels (a fertilizer response) and not only to the contribution of the seaweed extract (hormone-like stimulation), as P is known to enhance root growth (Anghinoni and Barber 1984). The significantly higher concentrations of Ca, Mg and K recorded in Kelpak® were well below that of fertilizer formulation levels aimed to meet crop requirements (Verkleij 1992). At these relatively low levels of Ca, Mg and K, certain minerals may act as biostimulants under stress conditions that can enhance the functionality of existing elements

Table 5 Rooting response as produced by mung bean hypocotyls in reaction to a concentration range of three seaweed (*Ecklonia maxima*) products namely Afrikelp®, Basfoliar® Kelp and Kelpak®, following the method of Crouch and Van Staden (1991). A dose response curve for rooting ability was performed using indole-3-butyric acid (IBA)

Concentration (%)	1	2	5	10	20	50
Afrikelp®	17.0 b	20.7 b	27.7 ns	25.9 ns	35.2 ns	18.6 b
Basfoliar®	18.0 b	21.8 b	23.7	31.8	32.7	27.5 ab
Kelpak®	25.2 a	28.9 a	35.5	37.7	48.2	36.3 a
$P<$	0.0129	0.0345	0.0990	0.2152	0.0530	0.0464
IBA (mg L^{-1})	0	0.5	1.0	2.0	5.0	10.0
	16.9±1.66	17.2±1.65	21.0±3.11	23.7±2.70	29.6±1.80	37.7±3.83
IBA (mg L^{-1})	20.0	50.0	100.0			
	51.3±4.08	58.6±4.22	74.4±5.09			

Data are presented as mean±standard error of the mean; similar letters within a column are not significantly different at the 5 % level

ns not significant

for increased plant growth and development (Beckett and Van Staden 1989, 1990; Papenfus et al. 2013). The elevated sodium concentration in Kelpak® could be partly ascribed to the higher alginic acid levels found in this product after processing (Tables 2 and 3), as alginates exist as a sodium salt of alginic acid (Craigie 2011).

Alginic acid analyses of raw seaweed indicated lower levels in Kommetjie than Gansbaai, and stipes showed lower levels than fronds (Table 1). The seaweed origin, ratio of stipes to fronds, the percentage extract used in the final formulation as well as the extraction method employed all influence the alginic acid levels in the final product. Kelpak® revealed significantly higher alginic acid levels versus the other two products. The seaweed used for Afrikelp® production grows in the warmer waters of Gansbaai (ca. 15.8 °C) compared to the seaweed harvested for Kelpak® production from Kommetjie (ca. 13.3 °C) (Lötze 2012) which may have caused this difference in alginic acid levels. It is also possible that the efficiency of the cell bursting pressure differential and centrifugation method as applied for the Kelpak® production (Stirk and Van Staden 1997) can increase the concentrations of specific components such as alginates, compared to the CMP extraction process followed by Afrikelp®, confirming findings by Craigie (2011). A product with a significantly higher alginic acid level may perform better than products with lower alginic acid levels when applied to soil.

Kelpak® contained significantly higher mannitol levels than Afrikelp® and Basfoliar® Kelp. Again, this may be due to the different growing conditions of the source material and possible variations in the processing methods.

Higher mannitol levels in the final product may be beneficial under stress conditions. Seckin et al. (2009) reported on increasing antioxidant enzyme activity in salt stress wheat after external mannitol application. A mannitol pre-treatment in these experiments resulted in increased activity of peroxidase, catalase and ascorbate peroxidase in wheat roots after exposure to salt conditions. The external mannitol treatment protected the roots against lipid peroxidation under these stress conditions. With respect to mineral absorption and translocation, boron absorption by soybean leaves was also shown to increase 24.5 % with addition of exogenous mannitol in the solution (Will et al. 2011). As boron has also been implicated in antioxidant formation, an increase in boron may further assist to reduce plant stress (Cakmak and Römheld 1997). Biostimulants are often recommended for use on plants that are under stress conditions, and products with higher mannitol content could contribute towards better root survival.

Amino acids play a fundamental role in protein synthesis in plants and are translocated between different plant organs, both in the xylem and phloem. Amino acids in Kelpak® were higher than in Afrikelp® and Basfoliar® Kelp (Table 4). As amino acids can also be acquired through the roots, additional available amino acids in the soil as would occur with the application of these biostimulants could promote mineral metabolism in general, such as the redistribution of nitrogen (Kielland 1994; Dakora and Phillips 2002).

The mung bean assay is an indirect quantification of plant growth regulator (hormonal) contents via the product's stimulation of root growth, usually associated with the presence of auxin-like substances (Crouch and Van Staden 1991). This assay showed significantly more or similar root formation with Kelpak® than the other two products at the concentrations tested. When seaweed products are applied with the specific aim to stimulate root growth, a product with higher auxin-like activity is likely to outperform similar products with lower levels of auxin-like activity, when applied at the same concentration.

From this study, it is evident that using the same source of seaweed species (*E. maxima*) for the manufacturing of seaweed extract for final commercial use does not guarantee a similar composition of available products. Season, location, manufacturing protocols, enrichment and percentage extract used in the product all impact on the final composition of the commercial product. The abovementioned differences in the composition of the three seaweed products (Afrikelp®, Basfoliar® and Kelpak®) may have varying results, even if applied under the same conditions, if used as similar products. It is therefore important that the composition of the products is clearly specified and disclosed on the product labels. Users should be informed prior to purchase on how differences between products would impact on the suitability for specific crops and applications.

Conflict of interest The authors hereby declare no conflict of interest.

References

Anghinoni I, Barber SA (1984) Phosphorus influx and growth characteristics of corn roots as influenced by phosphorus supply. Am Soc Agron 72:685–688

Arioli T, Mattner SW, Winberg PC (2015) Applications of seaweed extracts in Australian agriculture: past, present and future. J Appl Phycol. doi:10.1007/s10811-015-0574-9

Basak A (2008) Effect of preharvest treatment with seaweed products, Kelpak® and Goëmar BM 86® on fruit quality in apple. Int J Fruit Sci 8(1–2):1–14

Beckett RP, van Staden J (1989) The effect of seaweed concentrate on the growth and yield of potassium stressed wheat. Plant Soil 116:29–36

Beckett RP, van Staden J (1990) The effect of seaweed concentrate on the yield of nutrient stressed wheat. Bot Mar 33:147–152

Bohnert HJ, Jensen RG (1996) Metabolic engineering for increased salt tolerance—the next step. Aust J Plant Physiol 23:661–667

Bolton JJ, Anderson RJ (1990) Correlation between intertidal seaweed community composition and sea water temperature patterns on a geographic scale. Bot Mar 33:447–457

Briceño-Domínguez D, Hernández-Carmona G, Moyo M, Stirk W, van Staden J (2014) Plant growth promoting activity of seaweed liquid

extracts produced from *Macrocystis pyrifera* under different pH and temperature conditions. J Appl Phycol 26:2203–2210
Cakmak I, Römheld V (1997) Boron deficiency-induced impairments of cellular functions in plants. Plant Soil 193:71–83
Calvo P, Nelson L, Kloepper JW (2014) Agricultural uses of plant biostimulants. Plant Soil 383:3–41
Colavita GM, Spera N, Blackhall V, Sepulveda GM (2011) Effect of seaweed extract on pear fruit quality and yield. Acta Horticult 909: 601–607
Craigie JS (2011) Seaweed extract stimuli in plant science and agriculture. J Appl Phycol 23:371–393
Crouch IJ, Van Staden J (1991) Evidence for rooting factors in a seaweed concentrate prepared from *Ecklonia maxima*. J Plant Pathol 137: 319–322
Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil 245:35–47
Du Jardin P (2012) The science of plant biostimulants—a bibliographic analysis. ec.europa.eu/enterprise/sectors/…/files/…/final_report_bio_2012_en.pdf
DuBois M, Gilles KA, Hamilton JK, Rebers JK, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356
Featonby-Smith BC, Van Staden J (1988) The effect of seaweed concentrate on the growth of tomato plants in nematode-infested soil. Sci Hortic 20:137–146
Filisetti-Cozzi TMCC, Carpita NC (1991) Measurement of uranic acids without interference from neutral sugars. Anal Biochem 197:157–162
González A, Castro J, Vera J, Moenne A (2013) Seaweed oligosaccharides stimulate plant growth by enhancing carbon and nitrogen assimilation, basal metabolism, and cell division. J Plant Growth Regul 32:443–448
Grobbelaar MC, Makunga NP, Stander MA, Kossmann J, Hills PN (2014) Effect of strigolactones and auxins on growth and metabolite content of *Sutherlandia frutescens* (L.) R. Br. microplants in vitro. Plant Cell Tissue Organ Cult 117:401–409
Haug A, Larsen B (1962) Quantitative determination of uronic acid composition of alginates. Acta Chem Scand 16:1908–1918
Khan W, Zhai R, Souleimanov A, Critchley AT, Smith DL, Prithiviraj B (2012) Commercial extract of *Ascophylum nodosum* improves root colonization of alfalfa by its bacterial symbiont *Sinorhizobium meliloti*. Commun Soil Sci Plant 43:2425–2436
Kielland K (1994) Amino acid absorption by arctic plants: implications for plant nutrition and nitrogen cycling. Ecology 75:2373–2383
Lötze E (2012) A comparison between two, locally produced seaweed biostimulants regarding nutrient and alginic acid composition and biological rooting response. SA Fruit J 11(4):70–72
Manley SL (1981) Iron uptake and translocation by *Macrocystis pyrifera*. Plant Physiol 68:914–918
McGinley M (2011) Optimizing the analysis of sugar alcohol excipients in pharmaceutical tablet formulations using rezex ion exclusion HPLC columns. http://www.chromatographyonline.com/lcgc/Application+Notes%3A+Pharmaceutical/Optimizing-the-Analysis-of-Sugar-Alcohol-excipient/ArticleStandard/Article/detail/713164
Nayar S, Bott K (2014) Current status of global cultivate seaweed production and markets. World Aquacult 45:32–37
Papenfus HB, Stirk WA, Finnie JF, Van Staden J (2012) Seasonal variation in the polyamines of *Ecklonia maxima*. Bot Mar 55:539–546
Papenfus HB, Kulkarni MG, Stirk WA, Finnie JF, Van Staden J (2013) Effect of a commercial seaweed extract (Kelpak®) and polyamines on nutrient-deprived (N, P and K) okra seedlings. Sci Hortic 151: 142–146
Prabhavathi V, Rajam MV (2007) Mannitol-accumulating transgenic eggplants exhibit enhanced resistance to fungal wilts. Plant Sci 173:50–54
Seckin B, Sekmen AH, Turkan I (2009) An enhancing effect of exogenous mannitol on the antioxidant enzyme activities in roots of wheat under salt stress. J Pantl Growth Regul 28:12–20
Sharma SHS, Fleming C, Selby C, Rao JR, Martin T (2014) Plant biostimulants: a review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses. J Appl Phycol 26:465–490
Stirk WA, van Staden J (1997) Comparison of cytokinin- and auxin-like activity in some commercially used seaweed extracts. J Appl Phycol 8:503–508
Stirk WA, Tarkowski D, Turečová V, Strnad M, van Staden J (2014) Abscisic acid, gibberellins and brassinosteriods in Kelpak®, a commercial seaweed extract made from *Ecklonia maxima*. J Appl Phycol 26:561–567
Stoop JMH, Williamson JD, Pharr DM (1996) Mannitol metabolism in plants: a method for coping with stress. Trends Plant Sci 1:139–144
Tabarsa M, Karnjanapratum S, Cho M, Kim J, You S (2013) Molecular characteristics and biological activities of anionic macromolecules from *Codium fragile*. Int J Biol Macromol 59:1–2
Vera J, Castro J, Gonzalez A, Moenne A (2011) Seaweed polysaccharides and derived oligosaccharides stimulate defense responses and protection against pathogens in plants. Mar Drugs 9:2514–2525
Verkleij FN (1992) Seaweed extracts in agriculture and horticulture: a review. Biol Agric Hortic 8:309–324
Will S, Eichert T, Fernandez V, Möhring J, Muller T, Römheld V (2011) Absorption and mobility of foliar-applied boron in soybean as affected by plant boron status and application as a polyol complex. Plant Soil 344:283–293
Zodape ST (2001) Seaweed as biofertilizer. J Sci Ind Res 60:378382