UNIVERSIDADE DE CAXIAS DO SUL
ÁREA DE CONHECIMENTO DE CIÊNCIAS DA VIDA
INSTITUTO DE BIOTECNOLOGIA
PROGRAMA DE PÓS GRADUAÇÃO EM BIOTECNOLOGIA

EXTRATO AQUOSO DE *Kappaphycus alvarezii* CULTIVADA EM SANTA CATARINA: CARACTERIZAÇÃO BIOQUÍMICA SAZONAL E POTENCIAL BIOESTIMULANTE AGRÍCOLA EM CEVADA

Felipe de Souza Dutra

CAXIAS DO SUL
2026

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Tese apresentada ao Programa de Pós-graduação em Biotecnologia da Universidade de Caxias do Sul, como parte dos requisitos para a obtenção de grau de Doutor em Biotecnologia.

Orientador: Prof. Dr. Marcelo Marschin
Co-Orientador: Prof. Dr Sidnei Moura e Silva

CAXIAS DO SUL
2026

Dados Internacionais de Catalogação na Publicação (CIP)
Universidade de Caxias do Sul
Sistema de Bibliotecas UCS - Processamento Técnico

D978e Dutra, Felipe de Souza

Extrato aquoso de *Kappaphycus alvarezii* cultivada em Santa Catarina [recurso eletrônico] : caracterização bioquímica sazonal e potencial bioestimulante agrícola em cevada / Felipe de Souza Dutra. – 2026.

Dados eletrônicos.

Tese (Doutorado) - Universidade de Caxias do Sul, Programa de Pós-Graduação em Biotecnologia, 2026.

Orientação: Marcelo Marschin.

Coorientação: Sidnei Moura e Silva.

Modo de acesso: World Wide Web

Disponível em: https://repositorio.ucs.br

1. Biotecnologia marinha. 2. Bioquímica. 3. Agricultura sustentável. 4. Cevada. I. Marschin, Marcelo, orient. II. Silva, Sidnei Moura e, coorient. III. Título.

CDU 2. ed.: 606:574.5.08

Catalogação na fonte elaborada pela(o) bibliotecária(o)
Carolina Machado Quadros - CRB 10/2236

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Aprovado em _____de _____de 2026

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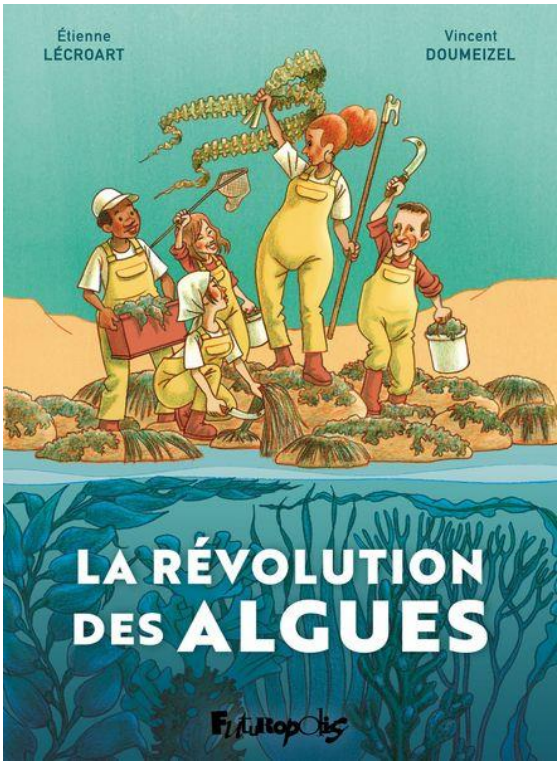

Capa do livro La Révolution des Algues (2022), de Vincent Doumeizel (autor) e Étienne Lécroart (ilustrador), publicado pela Éditions des Équateurs

"*E se as algas fossem o futuro do homem? Diante das crises demográfica, econômica e climática, esse tesouro esquecido bem poderia ser a solução*."
– Vincent Doumeizel

Agradecimentos

Ao meu orientador, Prof. Dr. Marcelo Maraschin, agradeço a confiança depositada desde o início. Obrigado por abrir as portas do laboratório e por me conceder autonomia para explorar ideias, mas sempre com o olhar atento e a palavra precisa nos momentos em que foi necessário redirecionar a rota. Levo comigo não apenas o conhecimento científico, mas o exemplo de liderança tranquila e generosa.

Ao meu coorientador, Prof. Dr. Sidnei Moura e Silva, agradeço a disponibilidade em integrar formalmente este projeto, estabelecendo uma ponte institucional entre a UCS e a UFSC que viabilizou esta parceria. Mesmo distante, esteve acessível para trocarmos ideias quando necessário.

À Regina, Alex, Aline e Gadiel, por me apoiarem e ensinarem com paciência e rigor, os fundamentos das análises bioquímicas e rotina de laboratório. Pela disposição para ouvir minhas perguntas, ideias de ensaios e desabafos! Cada extração, cada leitura de placa, cada curva de calibração trouxe consigo uma lição que ia além da bancada. Obrigado por não medirem esforços para que eu compreendesse os porquês por trás de cada protocolo. A dedicação de vocês foram pilares sobre os quais esta tese se ergueu.

A todos do Laboratório de Metabolômica Aplicada, que de alguma forma contribuíram para este trabalho e com a minha formação, seja com uma mão emprestada para finalizar um ensaio, uma palavra de incentivo em um dia difícil, as reuniões na incubadora fora de expediente, idas ao supermercado e noites de Magic! A ciência se faz em comunidade, e tive a sorte de estar cercado por pessoas generosas. São amigos que a pós-graduação me deu e que levo para a vida.

À Profª Drª. Joséli, à Drª Luciana, a todos os integrantes do Laboratório de Biotecnologia Vegetal da UCS e à Drª Deise, obrigado pelo acolhimento caloroso durante o período em que estive em Caxias do Sul. A parceria entre laboratórios foi fundamental. Que esta colaboração entre a serra gaúcha e o litoral catarinense perdure e gere muitos frutos.

À Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI), em especial ao Dr. Alex Alves dos Santos e a toda a equipe do Centro de Desenvolvimento em Aquicultura e Pesca (CEDAP), pelo suporte logístico e às empresas AlgasBrasil e VerdeMAr que gentilmente forneceram as amostras de extrato de *Kappaphycus alvarezii* e biomassa utilizadas neste estudo. Sem esta ponte entre a pesquisa básica e o setor produtivo, esta tese simplesmente não existiria. Que nossa colaboração continue a gerar inovação para a maricultura catarinense.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela bolsa de doutorado concedida (Processo nº 88887.696139/2022-00), que viabilizou minha dedicação integral a esta pesquisa.

À Universidade Federal de Santa Catarina (UFSC) e à Universidade de Caxias do Sul (UCS), pela infraestrutura e pelo ambiente acadêmico de excelência. Ao Programa de Pós-Graduação em Biotecnologia da UCS, nas pessoas de seus coordenadores, professores e servidores técnico-administrativos, pela dedicação em formar doutores preparados para os desafios da ciência brasileira.

À minha família, pelo apoio incondicional, pela compreensão nos momentos de ausência e por sempre acreditarem que este sonho era possível. Esta tese também é de vocês.

Muito obrigado!

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ABA	Ácido abscísico / Abscisic acid
AIA	Ácido indol-3-acético (Auxina) / Indole-3-acetic acid
BC.4	Modelo de Brain-Cousens de quatro parâmetros / Brain-Cousens four-parameter model
CAGR	Taxa de crescimento anual composta / Compound Annual Growth Rate
Ca	Cálcio / Calcium
CEDAP	Centro de Desenvolvimento em Aquicultura e Pesca (Epagri)
Chl a	Clorofila a / Chlorophyll a
Chl b	Clorofila b / Chlorophyll b
CO_2	Dióxido de carbono
Cond	Condutividade elétrica / Electrical conductivity
CONAB	Companhia Nacional de Abastecimento
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
Cu	Cobre / Copper
DM	Massa seca / Dry mass
DW (dw)	Peso seco / Dry weight
e / ED50	Dose efetiva 50% / Effective Dose 50
EPAGRI	Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina
FA	Ácido graxo / Fatty acid
FAO	Organização das Nações Unidas para Alimentação e Agricultura / Food and Agriculture Organization
Fe	Ferro / Iron
FM	Massa fresca / Fresh mass
FTIR	Espectroscopia de infravermelho com transformada de Fourier / Fourier-transform infrared spectroscopy
FW	Peso fresco / Fresh weight
GA / GA_3	Ácido giberélico / Gibberellic acid

GC-MS	Cromatografia gasosa acoplada à espectrometria de massa / Gas chromatography-mass spectrometry
GS	Taxa de granação / Grain set
H	Estatística de Kruskal-Wallis
HPLC	Cromatografia líquida de alta eficiência / High-performance liquid chromatography
INMET	Instituto Nacional de Meteorologia
IPCC	Painel Intergovernamental sobre Mudanças Climáticas / Intergovernmental Panel on Climate Change
K	Potássio / Potassium
LC-MS/MS	Cromatografia líquida acoplada à espectrometria de massa em tandem
MAPA	Ministério da Agricultura, Pecuária e Abastecimento
Mg	Magnésio / Magnesium
Mn	Manganês / Manganese
N	Nitrogênio / Nitrogen
Na	Sódio / Sodium
NDF	Fibra em detergente neutro / Neutral detergent fiber
NIR	Espectroscopia de infravermelho próximo / Near-infrared spectroscopy
NKP	Número total de grãos por vaso / Total kernels number per pot
NKS	Número de grãos por espiga / Number of kernels per spike
NUE	Eficiência do uso de nitrogênio / Nitrogen use efficiency
P	Fósforo / Phosphorus
PAL	Palhoça (local de coleta / sampling site)
PAL	Fenilalanina amonialiase / Phenylalanine ammonia-lyase
PC1, PC2, PC3	Primeiro, segundo e terceiro componente principal / First, second, third principal component
PCA	Análise de componentes principais / Principal component analysis
PH	Altura de planta / Plant height
ppb	Partes por bilhão

ppm	Partes por milhão
R^2	Coeficiente de determinação
RIB	Ribeirão da Ilha, Florianópolis (local de coleta / sampling site)
RMN	Ressonância magnética nuclear / NMR — Nuclear Magnetic Resonance
ROS	Espécies reativas de oxigênio / Reactive oxygen species
S	Enxofre / Sulphur
SA	Ácido salicílico / Salicylic acid
SC	Santa Catarina
SST	Temperatura da superfície do mar / Sea surface temperature
SVI	Índice de vigor de plântulas / Seedling vigor index
TAA	Aminoácidos totais / Total amino acids
TC	Carboidratos totais / Total carbohydrates
TCN	Carotenoides totais / Total carotenoids
TFC	Compostos flavonoides totais / Total flavonoid compounds
TKW	Peso de mil grãos / Thousand kernel weight
TL	Lipídios totais / Total lipids
TP	Proteínas totais / Total proteins
TPC	Compostos fenólicos totais / Total phenolic compounds
TS	Amido total / Total starch
TSC	Conteúdo de sólidos totais / Total solid content
TSP	Total de espigas por vaso / Total spikes per pot
TSS	Açúcares solúveis totais / Total soluble sugars
TTP	Total de afilhos por vaso / Total tillers per pot
UV-Vis	Espectroscopia ultravioleta-visível / Ultraviolet-visible spectroscopy
v/v	Volume por volume / Volume by volume
Zn	Zinco / Zinc

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Capítulo 3 – *Priming* em sementes de cevada (*Hordeum vulgare* L.)

	5, 10, and 20% v/v). Parameters: b = slope at inflection point; d = upper asymptote (response at maximal inhibitory concentration); e = ED50 (reference concentration dose); f = hormesis magnitude (suprabasal stimulation above upper asymptote; f > 0 indicates confirmed hormesis). Values presented as estimate ± SE with p-value in parentheses. r^2 = coefficient of determination for model goodness of fit. Statistically significant parameters: $p < 0.05$.

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N.º	Legenda
Fig. 1	Sample collection sites of the seaweed *Kappaphycus alvarezii* cultivated in sea farms in Florianópolis (RIB - Ribeirão da Ilha beach) and Palhoça (PAL) counties, state of Santa Catarina, southern Brazil. Source: Nunes et al. [19].
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	TC, total carbohydrates (mg g^{-1} dry matter); TSS, total soluble sugars (mg g^{-1} dry matter); TS, total starch (mg g^{-1} dry matter); TCN, total carotenoids (mg 100 g^{-1} dry matter); TPC, total phenolic compounds (mg 100 g^{-1} dry matter); TFC, total flavonoid compounds (mg 100 g^{-1} dry matter); Yield, carrageenan yield (%).
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	temperature; Ash, ash content; TP, total protein; TL, total lipids; NDF, neutral detergent fiber; TC, total carbohydrates; TSS, total soluble sugars; TS, total starch; TCN, total carotenoids; TPC, total phenolic compounds; TFC, total flavonoid compounds; Yield, carrageenan yield; N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; S, sulphur; B, boron; Cu, copper; Fe, iron; Mn, manganese; Zn, zinc.
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Capítulo 3 — *Priming* em Sementes de Cevada (*Hordeum vulgare*)

N.º	**Legenda**
Fig. 1	Germination percentage of barley (*Hordeum vulgare* L. cv. Rubi) seeds after priming with aqueous extract of *Kappaphycus alvarezii* at seven concentrations (0, 0.5, 1, 2, 5, 10 and 20% v/v). Bars represent means ± standard error. ns = no significant, by Dunn-Bonferroni post-hoc test ($p>0.05$).
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Fig. 3	Z-score heatmap of morphophysiological variables of barley seedlings in response to seed priming with *Kappaphycus alvarezii* biostimulant at seven concentrations (0, 0.5, 1, 2, 5, 10, and 20% v/v). Fresh shoot mass (FSM, mg), dry shoot mass (DSM, mg), fresh root mass (FRM, mg), dry root mass (DRM, mg), root:shoot ratio (R:S), shoot length (SL, cm), root length (RL, cm), and seedling vigor index (SVI). Numerical values within each cell represent the mean for the corresponding variable and concentration. Color scale indicates the Z-score relative to the grand mean across concentrations: red (warm) tones denote values above the cross-concentration mean ($Z > 0$), and blue (cool) tones denote values below the mean ($Z < 0$). Variables: fresh shoot mass (FSM; mg), dry shoot mass (DSM; mg), fresh root mass (FRM; mg), dry root mass (DRM; mg), root:shoot ratio (FRM:FSM), shoot length (SL; cm), root length (RL; cm), seedling vigor index (SVI).
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	Chl b = chlorophyll b (mg g^{-1}); TChl = total chlorophyll (mg g^{-1}); TCN = carotenoids (mg g^{-1}); TSS = total soluble sugars (mg g^{-1}); TS = total starch (mg g^{-1}); TP = total protein (mg g^{-1}); TAA = total amino acids (mg g^{-1}).

Capítulo 4 — Aplicação foliar do extrato aquoso de *Kappaphycus alvarezii* em plantas de cevada (*Hordeum vulgare* L.)

N.º	**Legenda**
Fig. 1	Yield components of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses diluted in water (control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil. A – grains set (GS); B – thousand kernel weight (TKW – g). Data are presented as mean + standard deviation. Values followed by different letters differ significantly for Dunn's test with Holm-Bonferroni adjustment ($p < 0.05$).
Fig. 2	Principal component analysis (PC1 and PC2) calculated from the data of plant height (PH), total tillers per pot (TTP), total spikes per pot (TSP), total kernels number per pot (NKP), number of kernels per spike (NKS), grain set (GS), and thousand-kernel weight (TKW), of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii*, at different doses (control, 1, 2, 5, and 10% v/v). The seaweed raw materials were cultivated in Santa Catarina, Brazil.
Fig. 3	Leaf pigments (mg g^{-1}) of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses diluted in water (control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil. A – chlorophyll a; B – chlorophyll b; C – total chlorophyll; D – total carotenoids (TCN). Values followed by different letters differ significantly for the Dunn's test with Holm-Bonferroni adjustment ($p < 0.05$).
Fig. 4	Biochemical parameters (mg g^{-1}) of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses diluted in water (0, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil. A – total carbohydrates (TC); B – total protein (TP); C – total amino acid (TAA). Values followed by different letters differ significantly for the Dunn's test with Holm-Bonferroni adjustment ($p < 0.05$).
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Fig. 6	Biochemical parameters (mg g^{-1}) of kernels produced by barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses (control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil. A – total soluble sugars (TSS); B – total starch (TS); C – total protein (TP); D – total amino acid (TAA). Values

	followed by different letters differ significantly for the Dunn's test with Holm-Bonferroni adjustment ($p < 0.05$).
Fig. 7	Principal component analysis (PC1 and PC2) calculated from the dataset of kernel germination (G%) and biochemical components: total starch (F1-TS), total soluble sugars (TSS) total protein (TP), and total amino acids (TAA), of barley plants treated with foliar application of aqueous extract of *K. alvarezii* at different doses (control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil.

Resumo

A busca por insumos agrícolas sustentáveis tem direcionado o interesse para bioestimulantes derivados de macroalgas marinhas. Neste contexto, esta tese objetivou caracterizar extratos comerciais de *Kappaphycus alvarezii* cultivada em fazendas marinhas no litoral de Santa Catarina e avaliar seu potencial bioestimulante em plantas de cevada (*Hordeum vulgare* L.), bem como investigar a composição e as potenciais aplicações do resíduo sólido da produção daquele bioinsumo. A primeira etapa consistiu na caracterização bioquímica do extrato comercial e de seu resíduo ao longo das estações, revelando uma influência marcante da sazonalidade sobre os perfis metabólicos daquelas biomassas. Amostras de extrato coletadas no inverno apresentaram teores significativamente superiores ($p<0,05$) de aminoácidos totais (0,52 a 6,90 mg/g), carboidratos totais (0,16 a 8,84 mg/g), açúcares solúveis (0,33 a 17,39 mg/g) e fenóis totais (2,22 a 23,17 mg/100g). Dentre os macronutrientes, o potássio mostrou-se em maior concentração (0,97% a 1,82%). A análise de componentes principais (PCA) discriminou claramente os perfis metabólicos das amostras de verão e inverno, com correlações negativas significativas entre a temperatura da superfície do mar e a concentração da maioria dos metabólitos investigados. O resíduo sólido, por sua vez, apresentou altos teores de cinzas (25,37 a 31,87% - peso seco), configurando uma matriz rica em minerais com maiores teores de Ca, Mg e S na primavera. Destaca-se ainda o rendimento expressivo de carragenana (média 17,87% - inverno) e a presença de compostos bioativos, incluindo carotenoides (até 248,96 mg/100g - inverno), flavonoides (até 13,54 mg/100g - outono) e ácidos graxos como o ácido heptadecanoico (C17:0, até 80,2% - inverno), conferindo potencial nutracêutico ao resíduo. Em conjunto, esses achados fundamentam um modelo de biorrefinaria em cascata sazonalmente orientado. Na segunda etapa, o potencial bioestimulante do extrato aquoso de *K. alvarezii* foi validado em sementes e plantas de cevada. No *priming* de sementes, o modelo de Brain-Cousens revelou uma janela de resposta hormética ótima entre concentrações de 2 e 5% (v/v). Nessa faixa, a massa fresca da parte aérea atingiu um incremento de 35% (5%, $r^2 = 0,99$) e a massa fresca radicular foi saturada já a 0,5% (+41%). Os teores de clorofilas a e b e carotenoides apresentaram maior conteúdo em plantas tratadas a 2% (~ +20%), seguindo um perfil unimodal. O conteúdo de amido total declinou monotonicamente em 59% entre o controle e o tratamento a 10%, enquanto a razão amido:proteína caiu de 6,31 ± 0,28 para 1,95 ± 0,50 (BC.4: $r^2 = 0,86$), refletindo uma transição progressiva do metabolismo de armazenamento de carbono para o anabolismo proteico. Em casa de vegetação, a aplicação foliar não alterou a arquitetura das plantas, mas promoveu melhorias significativas na qualidade dos grãos, com aumentos de até 19,3% nos teores de amido (5%), 10,3% de proteínas (10%) e 56,6% de aminoácidos totais (1 e 2%), mantendo a germinação acima de 98,5% em todos os tratamentos. Em conjunto, os resultados estabelecem uma base sólida entre a variabilidade sazonal da composição do extrato aquoso, sua atividade biológica hormética e o potencial de valorização integral da biomassa, posicionando o produto bioestimulante derivado de *K. alvarezii* cultivada no Sul do Brasil como uma ferramenta tecnológica confiável e sustentável à intensificação ecológica da agricultura, com capacidade de agregar valor à cadeia produtiva da aquicultura local.

Palavras-chave: biotecnologia marinha; caracterização bioquímica; Rhodophyta; agricultura sustentável; bioinsumos; *priming* de sementes; biorrefinaria; ácidos graxos.

Abstract

The search for sustainable agricultural inputs has driven interest in biostimulants derived from marine macroalgae. In this context, this thesis aimed to characterize the commercial extracts of *Kappaphycus alvarezii* cultivated in marine farms located on the coast of Santa Catarina state, southern Brazil, and to evaluate their biostimulant potential in barley (*Hordeum vulgare* L.), as well as to investigate the composition and potential applications of the solid residue from the production of this bioinput. The first stage involved the seasonal biochemical characterization of the commercial extract and its residue, revealing a marked influence of seasonality on the metabolic profiles of those biomasses. Extract samples collected in winter showed significantly higher ($p<0.05$) amounts of total amino acids (0.52 to 6.90 mg/g), total carbohydrates (0.16 to 8.84 mg/g), soluble sugars (0.33 to 17.39 mg/g), and total phenols (2.22 to 23.17 mg/100g), with potassium as the most abundant macronutrient (0.97% to 1.82%). Principal component analysis (PCA) clearly discriminated the metabolic profiles of summer- and winter-harvested samples, with significant negative correlations between sea surface temperature and the concentration of most metabolites. In turn, the solid residue retained high ash content (25.37–31.87%, dry weight), constituting a mineral-rich matrix with Ca, Mg, and S peaks in spring. Noteworthy are the expressive carrageenan yield (mean 17.87% – winter) and the presence of bioactive compounds, including carotenoids (up to 248.96 mg/100g – winter), flavonoids (up to 13.54 mg/100g – autumn), and fatty acids such as heptadecanoic acid (C17:0, up to 80.2% – winter), endowing the residue with nutraceutical potential. Taken together, these findings support a seasonally oriented cascading biorefinery model. In the second stage, the biostimulant potential of the aqueous extract was validated in barley seeds and plants. In seed priming, the Brain-Cousens model revealed an optimal hormetic window at 2–5% (v/v). Within this range, shoot fresh mass increased by 35% (5%, $r^2 = 0.99$) and root fresh mass was saturated at 0.5% (+41%). Chlorophylls a and b and carotenoids peaked at 2% (~ +20%), following a unimodal profile. Total starch content declined monotonically by 59% between the control and 10%, while the starch:protein ratio fell from 6.31 ± 0.28 to 1.95 ± 0.50 (BC.4: $r^2 = 0.86$), reflecting a progressive shift from carbon-storage to protein-anabolic metabolism. In the greenhouse, foliar application did not alter plant architecture, but significantly improved grain quality, with increases of up to 19.3% in starch (5%), 10.3% in proteins (10%), and 56.6% in total amino acids (1 and 2%), while maintaining germination above 98.5% in all treatments. Together, these results establish a solid foundation linking the seasonal variability of the aqueous extract composition, its hormetic biological activity, and the potential for full biomass valorization. The biostimulant product derived from the macroalga *K. alvarezii* cultivated in southern Brazil is thus positioned as a reliable and sustainable technological tool for the ecological intensification of agriculture, capable of adding value to the local aquaculture production chain.

Key-words: marine biotechnology; biochemical characterization; Rhodophyta; sustainable agriculture; bioinputs; seed priming; biorefinery; fatty acids.

1. Introdução

A intensificação agrícola baseada em insumos químicos sintéticos nas últimas décadas, na chamada Revolução Verde, gerou sérios problemas ambientais, como contaminação de solos e corpos d'água, perda de biodiversidade, degradação da matéria orgânica do solo e aumento da resistência de pragas e patógenos (Damalas & Koutroubas 2020; Tudi et al. 2021). Este cenário é agravado pelas mudanças climáticas, que impõem estresses abióticos cada vez mais severos às culturas (Raza et al. 2020; Zandalinas et al. 2021). Em resposta, surge a necessidade urgente de transição para sistemas agrícolas mais resilientes e ecológicos, capazes de conciliar produtividade com a preservação dos ecossistemas (Pretty et al. 2018).

Neste contexto, os bioinsumos ganham destaque central. Eles são definidos como produtos, processos ou tecnologias de origem biológica que atuam positivamente nos processos morfogenéticos e metabólicos das plantas (Brasil, 2020a). Este conceito alinha-se perfeitamente aos bioestimulantes, substâncias ou microrganismos aplicados para aumentar a eficiência nutricional, a tolerância a fatores de estresses (a)bióticos e a qualidade das culturas, independentemente de seu teor de nutrientes (du Jardin, 2015). Dentre estes, os extratos de algas marinhas emergem como uma das soluções mais promissoras, com capacidade de modular vias fisiológicas-chave nas plantas (Shukla et al. 2025).

Os extratos de algas atuam de forma multissetorial, estimulando o crescimento radicular e aéreo, aumentando a eficiência no uso de nutrientes e promovendo a tolerância a uma ampla gama de estresses, incluindo déficit hídrico, salinidade, extremos de temperatura - estresses abióticos, bem como ataques de fungos, bactérias e nematoides - estresses bióticos (Battacharyya et al. 2015; Ali, Ramsubhag & Jayaraman, 2021; Nunes et al. 2024a; Rabhi et al. 2025). Seu uso consolida-se, portanto, como uma ferramenta vital à construção de sistemas produtivos de baixa pegada de carbono, sendo plenamente compatível com os princípios da agricultura regenerativa e orgânica (Ali, Ramsubhag & Jayaraman. 2021; Singh et al. 2025; Shukla et al. 2025).

Extratos oriundos da macroalga vermelha *Kappaphycus alvarezii* apresentam um potencial significativo como bioestimulante, com diversos trabalhos demonstrando seus efeitos no aumento da produtividade agrícola e indução de respostas de defesa em plantas

(Nunes et al. 2024a, Shukla et al. 2025). Com a recente aprovação de seu cultivo em larga escala no Brasil (Brasil, 2020b), a caracterização detalhada e a validação da eficiência dos extratos de *K. alvarezii* já comercializados em culturas agrícolas torna-se um passo fundamental para garantir a qualidade do produto ao agricultor e consolidar este bioinsumo como uma ferramenta estratégica à agricultura nacional.

2. Justificativa

Devido à alta produção de biomassa, a espécie *K. alvarezii* produzida em SC possui grande potencial à exploração comercial. No entanto, sua utilização na agricultura ainda se mostra pouco explorada no Brasil. Neste contexto, a caracterização da composição bioquímica dos extratos aquosos, com alegado efeito bioestimulante do desenvolvimento e rendimento de espécies agrícolas, é uma demanda presente do setor privado produtor, decorrente das exigências ao registro de produtos. Assim, sabendo-se da bioatividade dos extratos algais na melhoria do crescimento e produção de plantas, faz-se necessário caracterizar e determinar a potencialidade dos bioestimulantes comerciais de *K. alvarezii* produzidos em SC, como estratégia de desenvolvimento de uma tecnologia agrícola promissora e de baixo impacto ambiental, subsidiando o registro de produtos comerciais nos órgãos competentes.

3. Objetivo Geral

Caracterizar bioquimicamente os extratos aquosos comerciais de *K. alvarezii* cultivada em Santa Catarina, e seu resíduo sólido, produzido em diferentes estações, e avaliar o seu potencial bioestimulante quando aplicado em sementes e plantas de cevada (*Hordeum vulgare* L.).

3.1. Objetivos Específicos

- Determinar a composição bioquímica e os teores de macro e micronutrientes de amostras coletadas ao longo das estações do ano de extratos aquosos comerciais de *K. alvarezii* cultivada em Florianópolis - SC e Palhoça - SC.
- Avaliar o perfil bioquímico e de macro e micronutrientes de amostras coletadas sazonalmente do resíduo sólido da produção dos extratos aquosos de *K. alvarezii* cultivada em Florianópolis - SC e Palhoça – SC, e discutir potenciais usos industriais.
- Avaliar o efeito de concentrações do extrato comercial de *K. alvarezii*, aplicado via *priming*, sobre a germinação, vigor e o metabolismo primário de sementes de cevada, por meio da determinação da taxa de germinação, comprimento e biomassa da parte aérea e radicular, teores de proteínas totais, aminoácidos totais e carboidratos totais, visando selecionar a dosagem mais promissora.
- Determinar, por meio de experimento de dose-resposta em casa de vegetação, a concentração mais adequada do extrato comercial de *K. alvarezii* ao cultivo de cevada, avaliando parâmetros morfológicos (crescimento e biomassa das plantas), componentes de produtividade e características bioquímicas das plantas e dos grãos produzidos.

4. Referencial Teórico

4.1.Histórico do uso de algas na agricultura.

O uso de algas pela civilização humana constitui uma prática ancestral com profundas raízes históricas, que antecedeu em milênios o advento da agricultura científica, sendo utilizadas para diversos fins por civilizações costeiras em escala global (Craigie, 2011; Erlandson et al. 2015). O registro mais antigo de interação entre humanos e algas marinhas remonta a cerca de 14.000 anos AP, documentado no sítio arqueológico de Monte Verde, no Chile. Lá, a descoberta de diversas espécies de algas associadas a fogueiras, ferramentas de pedra e resíduos alimentares mastigados sugere seu uso na dieta humana, provavelmente para fins medicinais – um uso que persiste entre populações indígenas locais até hoje (Dillehay et al. 2008). Este achado não apenas antecipou em milênios os primeiros registros documentais, mas também recalibrou as teorias de povoamento das Américas, fortalecendo a Hipótese do Corredor de Kelps, que postula como as florestas de macroalgas formaram um corredor costeiro de recursos do Japão ao Chile, sustentando migrações humanas que incluíam as algas como componente central da dieta marinha paleolítica (Erlandson et al. 2015). Esta longa tradição de utilização de recursos algáceos encontra paralelo na história oriental, onde o registro escrito mais antigo, originário da China há cerca de 1700 anos AP, documenta o uso de algas laminares da ordem Bangiales, tanto para fins alimentares quanto farmacêuticos, com diversas referências em textos antigos (Yang, Lu & Brodie, 2017), estabelecendo uma sucessão histórica transcontinental no aproveitamento destes recursos marinhos na saúde e alimentação humana (O'Connor, 2017).

Por sua vez, o emprego de algas marinhas como fertilizantes agrícolas possui raízes igualmente ancestrais, na região do Atlântico europeu. Os romanos foram os primeiros a documentar tal prática: L.J.M. Collumella, no século I d.C. (2000 AP), recomendava envolver raízes de mudas em algas para manter a umidade e preservar seu frescor (Handerson, 2004), enquanto Plínio, em 79 d.C., notava a coleta de margo (provavelmente maerl, uma alga vermelha) por povos da Britânia e Gália para fertilizar solos (Monagail et al. 2017). Evidências indicam, no entanto, que esta prática era anterior ao domínio romano, sendo já utilizada pelos povos autóctones do litoral Atlântico europeu (região da Bretanha). De acordo com os costumes locais, as macroalgas eram incorporadas diretamente à areia ou ao solo, ou então compostadas juntamente com palha,

turfa e outros resíduos orgânicos para aplicação posterior. Uma técnica amplamente utilizada consistia em amontoar as algas em campos e revolvê-las periodicamente num processo conhecido como decomposição controlada, um método que promovia a oxigenação do material e reduzia a formação de compostos sulfurados tóxicos (Craigie, 2011). Essa prática consolidou-se entre povos costeiros do Atlântico, que utilizavam algas regularmente para enriquecer solos agrícolas, difundindo-se por regiões como Islândia, Escandinávia, Península Ibérica e ilhas do Mediterrâneo. Em Portugal, por exemplo, a coleta de sargaço representava a mais importante atividade agro-marítima na Península Ibérica, integrando agricultura e pesca em um sistema harmonioso de aproveitamento de recursos costeiros (Pereira & Cotas, 2019). Estes agricultores ancestrais observaram consistentemente que o uso de algas conferia às culturas maior vigor, resiliência e produtividade, estabelecendo de forma intuitiva os princípios fundamentais que só séculos depois seriam validados pela bioquímica e fisiologia vegetal (Craigie, 2011).

Contudo, havia uma limitação logística de transportar algas *in natura* para além de poucos quilômetros do litoral, o que impulsionou, com o passar dos anos, o desenvolvimento de extratos alcalinos de macroalgas como solução tecnológica. Este avanço culminou em 1949 com a criação do primeiro método eficaz de liquefação de biomassa de algas para aplicação agrícola, representando um marco no aproveitamento econômico desses recursos (Craigie, 2011). Este desenvolvimento permitiu a expansão comercial em escala global, transformando os extratos de algas em um componente vital da agricultura moderna. Dessa forma, iniciou-se na segunda metade do século XX a fase de uso científico e comercial destes extratos de algas, consolidando esta inovação técnica. Entre estes, destacaram-se especialmente a partir dos anos 1950 produtos derivados de *Ascophyllum nodosum* (ex.: Maxicrop®, Kelpak®, Acadian®), na Europa e América do Norte (Shukla et al. 2019). Nas décadas seguintes, outras espécies ganharam relevância: *Durvillaea potatorum* e *Durvillaea antarctica* (Austrália e Chile), *Ecklonia maxima* (África do Sul), *Sargassum* spp. e *Undaria pinnatifida* (Ásia), *Macrocystis pyrifera* (Califórnia e Chile), além de algas vermelhas como *Gracilaria* spp. e *Chondrus crispus* (Craigie, 2011; Sharma et al. 2014).

A elucidação científica de componentes bioativos específicos em extratos de algas marinhas acelerou o desenvolvimento e a sua adoção comercial. Pesquisas identificando citocininas, auxinas e betainas em extratos algais forneceram explicações mecanísticas

para os efeitos promotores de crescimento e mitigadores de estresse observados em ensaios a campo (Shukla et al. 2019; Ali, Ramsubhag & Jayraman, 2021). Paralelamente, o avanço nas técnicas analíticas foi crucial para elucidar a composição dos extratos. Métodos cromatográficos, como a cromatografia em fase gasosa acoplada à espectrometria de massa (GC-MS) e a cromatografia líquida de alta eficiência (HPLC), permitiram identificar e quantificar compostos orgânicos específicos, como fitormônios e aminoácidos (Das & Prasad, 2015; Vaghela et al. 2022; 2023a). Já as técnicas espectroscópicas, incluindo as espectroscopias de ressonância magnética nuclear (RMN), de infravermelho médio (FTIR), de infravermelho próximo (NIR) e de ultravioleta-visível (UV-Vis), forneceram *insights* sobre a estrutura molecular de polissacarídeos e outros constituintes (Ertani et al. 2018). Em conjunto, essas ferramentas viabilizaram um controle de qualidade rigoroso e a indispensável padronização dos bioestimulantes comerciais (Rabhi et al. 2025). Assim, na década de 1990, os bioestimulantes algais haviam estabelecido um mercado de nicho na agricultura sustentável, com adoção crescente em sistemas de agricultura orgânica onde as opções de insumos sintéticos eram limitadas (Craigie et al. 2011).

Já, no século XXI, a indústria de bioestimulantes algais evoluiu para a diversificação de produtos e estratégias de formulação sofisticadas (Rouphael & Colla, 2018; Shukla et al. 2025), como nanoformulações (e.g. Magnabosco et al. 2023; Patra, Shin & Das, 2025) e combinações dos extratos algais com outros ingredientes bioativos, como substâncias húmicas ou microrganismos benéficos (e.g. Ngoroyemoto, et al. 2020; Rinaldi et al. 2023), criando assim efeitos sinérgicos.

Atualmente, os extratos de algas marinhas são amplamente utilizados como bioestimulantes, movimentando um mercado multimilionário global avaliado em US$ 1,41 a 2,5 bilhões em 2025, com crescimento robusto (CAGR) de 4% a 13,6% até 2032-2034), impulsionado por pressões regulatórias sobre insumos químicos, transição para práticas agrícolas ecológicas e demanda por alimentos sustentáveis (GMI, 2025). O Brasil regulou o uso de algas na agricultura em 2020, através da Instrução Normativa 61 do Ministério da Agricultura e Pecuária (MAPA), que fixa regras para biofertilizantes e para o cultivo de *Kappaphycus alvarezii* nos estados de Santa Catarina, São Paulo e Rio de Janeiro (Brasil, 2020b). Mesmo recente no mercado, os extratos de algas já lideram o segmento de ingredientes ativos em bioinsumos, detendo 59% de participação em 2024,

com projeções de valores de comercialização avaliado entre US$ 51,29 milhões e US$ 73,5 milhões em 2024/2025 (Mordor Inteligence, 2025), impulsionados principalmente por programas governamentais como o Programa Nacional de Bioinsumos (Brasil, 2020a). Atualmente, estão registrados no mercado brasileiro 2581 produtos a base de extratos de algas, sendo divididos em fertilizantes orgânicos (1322), fertilizantes minerais (1265), biofertilizantes (4) e condicionador de solo (1). Em sua maioria, estes bioprodutos são derivados das algas *Ascophyllum nodosum*, oriunda de importação, e o complexo de algas do gênero *Lithothaminum* (Brasil, 2025), obtido por meio de extrativismo no litoral brasileiro (Paiva et al. 2023).

4.2.A macroalga *Kappaphycus alvarezii*

4.2.1. Biologia de *K. alvarezii*

K. alvarezii (Doty) L.M.Liao 1996: 158 é uma macroalga vermelha, pertencente à família Solieriaceae, ordem Gigartinales, filo Rhodophyta (AlgaeBase, 2025). É uma espécie perene, podendo viver até dois anos. Possui reprodução sexuada ou assexuada, com alternância de gerações, passando pelas fases esporofítica, gametofítica e carposporofítica. É nativa da região do Indo-Pacífico, alcançando o leste africano, a Índia e China, até as ilhas do Sudeste Asiático e Japão. Seus hábitats típicos são recifes de corais e zona do infralitoral. A alga desenvolve-se preferencialmente em águas rasas (1 – 17 m), quentes (27 – 30 °C), e transparentes, sendo adaptada a elevados níveis de radiação solar (Montúfar-Romero et al. 2023).

A espécie passou por várias revisões taxonômicas desde sua descrição original, refletindo o avanço na compreensão de suas relações filogenéticas. Historicamente classificada no gênero *Eucheuma* como *E. alvarezii*, foi subsequentemente reclassificada para o gênero *Kappaphycus*, em 1996*,* com base em características morfológicas, metabólicas e reprodutivas distintas, particularmente sua produção de *kappa*-carragena em vez de *iota*-carragena, típica de espécies do gênero *Eucheuma* (Liao, 1996). A correta determinação do status taxonômico é fundamental para o cultivo e aplicações industriais dessas algas. Contudo, espécies de *Eucheuma* e *Kappaphycus* são frequentemente identificadas erroneamente (Lim et al. 2017). Atualmente, são reconhecidas, taxonomicamente, seis espécies do gênero *Kappaphycus* e trinta do gênero *Eucheuma*,

sendo *K. alvarezii*, *K. striatus* e *E. denticulatum* as mais conhecidas devido ao seu valor comercial (Doty, 1988). A plasticidade morfológica e o uso generalizado de nomes comerciais, como "cottonii" para designar *Kappaphycus*, contribuem para identificações incorretas, agravadas pelo cultivo de populações mistas por maricultores (Lim et al. 2017). Coletivamente, esse grupo é referido como "eucheumatoides", e apesar da maioria ser identificada morfologicamente como *K. alvarezii* e *K. striatus*, observa-se a carência de confirmação da identidade genética, evidenciando a complexidade taxonômica envolvida (Neish & Suryanarayan, 2017).

Diante disso, a introdução de novas linhagens tem sido estudada para aumentar a produtividade, manter a qualidade e otimizar a produção de compostos bioativos. Narvarte et al. (2022) destacaram a importância de estudar linhagens selvagens de *K. alvarezii*, uma vez que cultivares antigos podem ser mais susceptíveis a estressores ambientais, como pragas e doenças. De fato, a maioria dos sistemas de aquicultura comercial de *K. alvarezii* no mundo é baseada em poucas linhagens, resultando em baixa variabilidade genética e maior susceptibilidade a fatores de estresse abióticos e bióticos. Portanto, a introdução de novas linhagens é essencial para melhorar o pool genético e o desempenho das variedades cultivadas, um ponto que também é enfatizado por Hinaloc e Roleda (2021). Outro fator que dificulta a sua correta identificação é a grande plasticidade morfológica que estas algas possuem.

A morfologia de *K. alvarezii* exibe variação considerável influenciada por diversas condições ambientais, com diferentes morfotipos de cor incluindo vermelho, marrom, amarelo e verde (Figura 1), dentre outras combinações de cores, de acordo com a concentração de ficoreritrina (Hinaloc & Roleda, 2021). Seus talos são cartilaginosos, cilíndricos e arbustivos com uma estrutura de crescimento multiaxial apresentando padrões de ramificação irregulares, às vezes opostos ou falsamente dicotômicos, variando de 24 a 48 cm de comprimento (Doty, 1988). As carragenanas são armazenadas predominantemente nas paredes celulares, contribuindo à textura cartilaginosa do talo (Rupert et al. 2022).

Figura 1 - *Kappaphycus alvarezii* cultivada em Santa Catarina. Sistema de cultivo tipo *tie-tie* (esquerda) e variação de cores de linhagens (direita). Fonte: Epagri.

4.2.2. Cultivo de *K. alvarezii*

K. alvarezii é a quinta macroalga mais cultivada globalmente, porém é a principal fonte de carragenana, contribuindo com cerca de 90% da produção mundial, junto *Eucheuma denticulatum*. A carragenana é um polissacarídeo com ação gelificante e espessante, muito utilizado nas indústrias alimentícia, farmacêutica e de cosméticos (Rudke, Andrade & Ferreira, 2020). Em 2022, foram produzidas 36,5 milhões de toneladas de algas no mundo e *K. alvarezii* contribuiu com 5% desta produção em biomassa fresca (FAO, 2024). Esta espécie apresenta rápido crescimento, de cerca de 4,5% de seu peso por dia, uma característica associada ao seu sucesso na aquicultura (Rudke, Andrade & Ferreira, 2020). Seu cultivo comercial foi inicialmente desenvolvido e consolidado nas Filipinas e na Indonésia, que se tornaram os centros históricos de produção. Todavia, devido ao seu alto valor econômico como fonte de carragenana, o cultivo expandiu-se para inúmeros outros países, incluindo Índia, Malásia, Tanzânia e China (Hurtado, Critchley & Neish, 2018).

No Brasil, *K. alvarezii* foi introduzida em 1995 por meio de uma parceria entre a Universidade de São Paulo e o Instituto de Pesca, para pesquisa e avaliação do seu potencial de bioinvasão, conteúdo de carragenana e biocombustível (Gelli et al. 2024). Esta introdução no Brasil ocorreu motivada pela crescente demanda por carragenana, na tentativa de tornar o Brasil autossuficiente na produção deste polissacarídeo (Oliveira, Silva & Amancio, 2009). O cultivo ganhou ímpeto após a autorização ambiental para seu cultivo comercial no Brasil em 2020, nos estados de São Paulo, Santa Catarina e Rio de Janeiro (Brasil, 2020a). Dentre estes, o estado de SC tem se destacado, emergindo como

um centro estratégico ao cultivo de *K. alvarezii* e a produção de extrato aquoso com alegadas propriedades bioestimulantes do desenvolvimento e produção vegetal. Em 2024, o estado catarinense produziu 751 t de biomassa algal fresca e 600.872 litros de bioestimulante, gerando aproximadamente US$ 1.991.840 em receita apenas com a venda do bioestimulante (Santos, 2024). Embora o cultivo comercial em larga escala desta alga vermelha ainda esteja em seus estágios iniciais, estes resultados demonstram o considerável potencial do Estado como uma região viável tanto para a aquicultura de *K. alvarezii* quanto para a valorização dos seus subprodutos (Hayashi et al. 2024).

4.2.3. Caracterização da composição de extratos aquosos de *K. alvarezii*

A produção de *K. alvarezii* se expandiu para além da produção de carragenana, passando a incluir também a produção de bioestimulantes. A metodologia para obtenção do extrato pode envolver um processo simples de extração aquosa, com trituração da biomassa algal fresca, seguida de filtração à recuperação do extrato aquoso (Layek et al.,2015; Nunes, 2025), até processos mais elaborados, como os homogenados minimamente processados (Vaghela et al., 2023a).

A composição dos extratos de *K. alvarezii* tem sido pouco explorada, apesar do crescente número de relatos na literatura. Vários metabólitos primários e secundários oriundo das algas podem ser encontrados nos extratos aquosos, e.g., carboidratos (5,70 – 5,88 g/100 g), proteínas (3,4 – 3,91 g /100 g), lipídios (2,13 – 2,66 g/100 g), carotenoides (0,64 – 4,26 g/100 g) e fenólicos totais (1108,67 – 1244,25 mg de ácido gálico (GA) /100 g) (Vaghela et al. 2022; 2023a). Fitormônios também têm sido identificados, a exemplo de giberelinas (14,31 – 27,11 ppm), auxinas (ácido indolacético – AIA, 1777 – 2514 ppb) e citocininas (zeatina - 568 – 2010 ppb) (Zodape et al. 2009; Vaghela et al. 2023a). Vaghela et al. (2023a) também identificaram aminoácidos, como glicina (1,01 mg/g), beta-alanina (0,97 mg/g), L-valina (0,4 mg/g) e L-cisteína (0,32 mg/g). Porém, os metabólitos mais abundantes foram tiramina (21,09 mg/g), formamida (14,93 mg/g) e D-(-)-eritrose (7,66 mg/g). Gandhi et al. (2024) realizaram uma caracterização aprofundada de metabolitos em extratos aquosos de *K. alvarezii*, identificando 198 compostos bioativos, incluindo glucose, glicina, valina, ácido elágico, genisteína e poliaminas.

Além dos metabólitos, os extratos também possuem macro e micronutrientes, sendo o potássio o mais abundante, com valores que variam de 16 a 33 g/L (Zodape et al., 2008; Rathore et al. 2009; Layek et al. 2015; Gelli et al. 2020). Os demais macronutrientes como nitrogênio, fósforo, cálcio, magnésio, apresentam valores <0,5 g/L, não sendo possível a sua utilização para suprir as necessidades nutricionais das plantas de interesse agronômico. Ao contrário do potássio, onde seu o uso do extrato como fonte de potássio já foi testado em cultivo de cana-de-açúcar (Karthikeyan & Shanmugam, 2017).

4.2.4. Efeitos promotores de crescimento e produtividade em plantas

Os efeitos bioestimulantes de extratos de *K. alvarezii* foram documentados em diversas culturas agrícolas, demonstrando melhorias significativas em parâmetros de crescimento e acúmulo de compostos bioativos (Trivedi et al. 2023). O primeiro artigo publicado sobre a aplicação de extrato de *K. alvarezii* em plantas é de Zodape et al. (2008), que relata um experimento de campo realizado no verão de 2006 em okra (*Abelmoschus esculentus* L.), aplicado como spray foliar em concentrações de 2,5%, 5,0%, 7,5% e 10,0%. Os resultados mostraram aumento significativo no rendimento (20,47% na concentração de 2,5%) e na qualidade nutricional dos frutos, atribuídos a microelementos e reguladores de crescimento vegetal presentes no extrato, marcando o início de estudos específicos sobre essa alga como biostimulante agrícola.

Em outros trabalhos, a aplicação foliar do extrato de *K. alvarezii* a 7,5% (v/v), promoveu o crescimento e a produtividade do arroz (Deb & Singh, 2022), além de melhorar atributos de qualidade física (descascamento, beneficiamento e rendimento) de grãos inteiros, características bioquímicas (teor de amilose e carboidratos) e a produtividade das plantas (Pramanick, Brahmachari & Mahapatra, 2020). De forma análoga, em outro estudo, o extrato foi aplicado juntamente com o extrato aquoso de *Gracilaria edulis*, via pulverização foliar a 15%, resultando em incremento significativo do rendimento de grãos de arroz e reduzindo a duração do ciclo de cultivo, evitando a exposição a eventuais fatores estressores ambientais (Sharma et al. 2017). Da mesma forma, avaliações da eficácia da pulverização foliar de ambos os extratos nas concentrações de 5%, 10% e 15% incrementaram o crescimento e a produtividade do

arroz. Adicionalmente, na concentração de 10%, observou-se um aumento nos teores de micronutrientes (Fe, Cu, Zn e Mn) e de proteínas nos grãos (Layek et al. 2018). Quando o extrato de *K. alvarezii* aplicado via pulverização foliar em plantas de arroz nas concentrações de 5% e 10%, houve aumento da massa seca das raízes e folhas A concentração de 10% também incrementou a área, o diâmetro e o número de raízes (Castro et al. 2023). Em aplicação via irrigação do solo a 3%, detectou-se estímulo à produção de massa fresca e seca das raízes, enquanto, a 2%, o bioinsumo promoveu aumento de área, comprimento, número e volume radicular, acompanhado de intensa mobilização e alocação de K^+ nas folhas, aumentando consequentemente a eficiência de absorção deste nutriente (Castro et al. 2023).

Em plantas de milho, a aplicação foliar do extrato de *K. alvarezii* aumentou a produtividade quando aplicada no estádio de desenvolvimento V15 ou posterior, com uma redução líquida de 20% no impacto relacionado às mudanças climáticas, atenuando o estresse ambiental sobre a cultura (Trivedi et al. 2017). Basavaraja et al. (2018) constataram aumento no crescimento e produtividade de grãos de milho com a aplicação conjunto dos extratos de *K. alvarezii* e *G. edulis* a 10%. Por sua vez, Vaghela et al. (2023b) descreveram que a aplicação foliar em plantas de milho de extratos aquosos de *K. alvarezii* em baixa concentração, i.e., 0,35% (v/v) resultou em incrementos no crescimento e produtividade, enquanto uma dose maior (0,7%) não promoveu incremento adicional.

Em plantas de batata, a aplicação foliar do extrato a 5,0% ou 7,5% (v/v) incrementou o crescimento, a produtividade e qualidade dos tubérculos (Pramanick et al. 2017). Aquele bioinsumo aplicado a 10%, juntamente com *G. edulis*, também promoveu o crescimento e produtividade, além de otimizar as propriedades físico-químicas das batatas (Garai et al. 2021).

Em cana-de-açúcar, observou-se aumento de produtividade e qualidade do mosto quando as plantas foram tratadas inicialmente com 0,3% (v/v) e, posteriormente, com 1% de do extrato de *K. alvarezii*. Tais melhorias podem estar relacionadas ao alto teor de potássio (11,28%) do extrato (Karthikeyan & Shanmugam, 2017). A concentração de 5% também aumentou a produtividade da cana-de-açúcar e reduziu as emissões de gases de efeito estufa (GEE) em pelo menos 260 kg de CO_2 equivalente por tonelada de cana produzida por hectare (Singh et al. 2018).

Outros estudos concentraram-se na análise do crescimento e produtividade de diferentes espécies. Em soja, por exemplo, sob condições de sequeiro e sem o uso de fertilizantes químicos, aplicações foliares de extrato de *K. alvarezii* em até 15% (v/v) aumentaram significativamente a altura da planta, número de ramos e folhas, comprimento e volume radicular, peso seco, atributos de rendimento (vagens/planta, sementes/vagem, peso de 100 sementes) e absorção de nutrientes (N, P, K e S), culminando em um incremento de 57% no rendimento de grãos e maior rendimento de palha comparativamente ao controle (Rathore et al. 2009). A aplicação foliar do extrato aquoso a 5% em tomateiros resultou em aumentos significativos no crescimento vegetativo (altura da planta em 34,44%, comprimento radicular em 45,05% e teor de clorofila em 53,85%), no rendimento (60,89% na produtividade de frutos e 75,11% no número de frutos por planta) e na absorção de nutrientes (até 61,92% para potássio), além de melhorias na qualidade dos frutos, como elevação de 23,81% no ácido ascórbico e nos sólidos totais (Zodape et al. 2011).

Ao testarem extratos de *K. alvarezii* e *G. edulis*, Dutta et al. (2019) verificaram que diferentes concentrações de ambos os extratos estimularam a germinação e incrementaram o tempo de armazenamento e a produção de frutos de pimenta (*Capsicum frutescens*). Um aumento no crescimento de plantas de amaranto (*Amaranthus polygamous*) foi descrito após tratamento com 50% de fertilizante comercial, suplementado com 10% (v/v) de extrato de *K. alvarezii*, bem como a elevação dos conteúdos foliares de N, P, K e Na (Senthuran et al. 2019). Em orquídeas da espécie *Epidendrum secundum* foram observados estímulos ao enraizamento, desenvolvimento de mudas e ganho de biomassa fresca, quando tratadas com 50 mg L^{-1} do extrato de *K. alvarezii* (Amatuzzi et al. 2020).

Além dos efeitos no crescimento, alguns trabalhos também demonstraram respostas metabólicas de plantas tratadas com extratos de *K. alvarezii*. Por exemplo, em condições de solos pobres em nutrientes no Nordeste da Índia, o extrato aplicado via foliar em plantas de milho aumentou o rendimento de grãos em 10,5-13,1%, o teor de carboidratos em 12,3-17,4% e de proteínas em 4,8% (Layek et al. 2015). Estacas de kiwi tratadas com soluções a 10% (v/v) de *K. alvarezii*, *G. edulis*, *A. nodosum*, *E. maxima*, ou ácido húmico apresentaram ganhos significativos na porcentagem de enraizamento, além de aumento nos parâmetros de crescimento, pigmentos (clorofila e carotenoides) e

metabólitos (carboidratos totais e fenóis solúveis), com redução no vazamento de eletrólitos (Dutta et al. 2023). Layek et al. (2023) observaram aumentos significativos no crescimento e produção de feijão-vagem, quiabo e tomate com aplicação foliar a 10% (v/v) de extratos comerciais de *K. alvarezii* e *G. edulis*, além de maior teor de ácido ascórbico e licopeno em tomates e maior atividade antioxidante. Shukla et al. (2023) demonstraram que o tratamento com extrato comercial de *K. alvarezii* (1 mL L^{-1}) aumentou em 29,2% a área foliar de cotilédones de pepino, com maior atividade de amilase, indicando conversão eficiente de amido em sacarose. Por fim, Nunes et al. (2025) verificaram que o tratamento foliar em plantas de manjericão (*Ocimum basilicum*) com biostimulante de *K. alvarezii*, em concentrações de 5-7% (v/v), aumentou significativamente as concentrações de clorofila (até 13,19% em Chl-a), carotenoides (até 20%), açúcares solúveis (até 185,81%), carboidratos totais (até 84,91%), aminoácidos totais (até 200%) e certas aminas biogênicas como putrescina e histamina, com efeitos variáveis por origem geográfica da alga.

4.2.5. Efeitos indutores de resposta de defesa de plantas a estressores ambientais

Efeitos positivos da aplicação de extratos aquosos algais em plantas, no que tange à redução dos efeitos de fatores de estresses abiótico e biótico também foram observados. Em plantas de milho submetidas a estresses hídricos moderado (14%) e severo (23%), tratadas por via foliar com o extrato de *K. alvarezii* a 10% houve aumento no rendimento de grãos e da concentração e atividade de sistemas antioxidantes enzimáticos e não enzimáticos (Trivedi et al. 2018). Em ensaio similar, aqueles autores observaram também uma maior produtividade em plantas de milho sob estresse hídrico tratadas nos estádios V5 e V15, com maiores valores de atividades de enzimas antioxidantes (Trivedi et al. 2022). Em trigo duro, sob estresse salino e hídrico, a aplicação do extrato melhorou parâmetros morfológicos, físico-bioquímicos e de expressão gênica, reduzindo a razão Na^+/K^+, danos à membrana celular e conteúdo de malondialdeído, enquanto promoveu incrementos do conteúdo de água tissular, proteínas totais, prolina, aminoácidos e açúcares solúveis (Patel et al. 2018). Vaghela et al. (2023a) observaram aumento da produtividade do tomate com aplicação de 0,8% de extratos de *K. alvarezii* e *S. wightii*, sob diferentes regimes hídricos.

O conhecimento dos mecanismos associados aos efeitos indutores de defesa de extratos de *K. alvarezii* em plantas ainda é escasso. Estudos transcriptômicos em milho revelaram a ativação de genes induzidos pela aplicação do extrato algal. Por exemplo, plantas tratadas com o extrato aquoso em ambiente com déficit hídrico apresentaram genes diferencialmente expressos envolvidos em síntese proteica, de parede celular, sinalização, transporte, estresse, desenvolvimento, metabolismo secundário, metabolismo hormonal, DNA, metabolismo lipídico e vias relacionadas ao metabolismo de N, S-transferases e peroxidases, com regulação negativa de transcritos relacionados à degradação de amido e sacarose (Kumar et al. 2020a). Trivedi et al. (2021a) detectaram alteração no padrão de expressão gênica, com 380 genes up-regulados e 631 down-regulados após a aplicação do extrato aquoso de *K. alvarezii* no estádio V5 do milho, sob estresse hídrico. Os genes com incremento de expressão mostraram-se envolvidos no transporte de nitrato e outros íons, fotossíntese, biossíntese de glicogênio e amido e sinalização de fitormônios.

Em relação a estresses bióticos, causados principalmente por fitopatógenos, há dois mecanismos pelos quais os extratos parecem atuar, conferindo proteção contra patógenos, sendo mediados por um sistema de detecção em duas camadas (Jones & Dang, 2006; Thomma, Nürnberger & Joosten, 2011). A primeira camada é ativada quando receptores de reconhecimento de padrões (PRRs) na superfície celular detectam elicitores microbianos, desencadeando uma cascata de sinalização de defesa. A segunda camada ocorre intracelularmente quando proteínas R detectam efetores patogênicos, amplificando drasticamente as respostas de defesa (Thomma, Nürnberger & Joosten, 2011). As respostas celulares incluem a produção de espécies reativas de oxigênio (ROS) e a modulação de hormônios, como o ácido salicílico (SA). Outros fitormônios, como auxina, ácido abscísico e citocininas, atuam de forma sinérgica ou antagônica para ajustar finamente essa resposta (Bari & Jones, 2009).

Notavelmente, extratos de algas, particularmente seus polissacarídeos, podem induzir respostas de defesas em plantas, ativando vias de sinalização do SA e/ou do jasmonato de maneira dependente do composto e da espécie vegetal (Stadnik & Freitas, 2014). Melo et al. (2020) confirmaram a ação indutora do extrato de *K. alvarezii* em atividades enzimáticas de tomateiros infectados com *Fusarium oxysporum*, apresentando os maiores índices de β-1,3-glucanase, peroxidase e fenilalanina amonialiase (PAL).

Também foi observado que o extrato aquoso (100 μg mL^{-1}) apresentou atividade contra *Bacillus subtilis*, *Streptococcus aureus*, *Lactobacillus acidophilus*, *Escherichia coli*, *Pseudomonas aeruginosa* e *Proteus mirabilis* (Banu, Ramani & Murugan, 2020). Neste mesmo estudo, o extrato a 3% aplicado sobre frutos de tomate, aumentou a qualidade dos frutos durante o armazenamento, provavelmente via atividades indiretas – indutores de defesa nos frutos – ou diretas, i.e., atividade microbiana (Banu, Ramani & Murugan, 2020). Por fim, Roy et al. (2022) verificaram aumento da atividade de defesa contra *Xanthomonas oryzae* em *Arabidopsis thaliana* e arroz após aplicação foliar do extrato, devido ao aumento nos teores de ácido salicílico, ácido jasmônico e citocinina, após a aplicação de dois formulados com extratos de *K. alvarezii*: Tomatough® (4 mL L^{-1}) e AgFort® (1 mL L^{-1}). Além disso, a própria carragenana isolada de *K. alvarezii* demonstrou efeitos indutores de defesa em plantas. Por exemplo, a aplicação foliar de κ-carragenana induziu a enzima antioxidante peroxidase em folhas de pimenta, regulou 17 proteínas e apresentou efeito fungistático *in vitro* contra *Colletotrichum gloeosporioides*, reduzindo o índice da doença nas plantas (Mani e Nagarathnam, 2018). Mani et al. (2021) demonstraram que κ-carragenana extraída de *K. alvarezii* induziu resposta de resistência em tomateiros ao controle da mancha foliar causada por *Septoria lycopersici,* com redução da doença, indução de H_2O_2 e ânion superóxido, aumento da atividade de peroxidase e regulação positiva de 11 proteínas. Tais evidências demonstram, portanto, que os extratos de *K. alvarezii* atuam como elicitores multifacetados, capazes de induzir um estado de resistência sistêmica nas plantas.

4.2.6. Efeitos moduladores da microbiota do solo

A microbiota do solo representa outro alvo para efeitos mediados por extratos de *K. alvarezii*, embora essa área tenha recebido menos atenção da pesquisa, comparativamente aos estudos de produtividade e indução de respostas de defesa de plantas. Evidências sugerem que a aplicação de extratos de algas pode influenciar a composição e função da comunidade microbiana na rizosfera, potencialmente aprimorando interações benéficas planta-micróbio (Ali, Ramsubhag & Jayaraman, 2021). Trivedi et al. (2021b) demonstraram que a aplicação do extrato de *K. alvarezii* aumentou a diversidade bacteriana e as atividades enzimáticas do solo, com melhorias no rendimento das plantas de milho, pelo escorrimento das folhas no solo. A aplicação foliar

de extrato comercial de *K. alvarezii* (AgroGain/LBS6), além de melhorar a morfologia e processos fisiológicos das plantas, foi capaz de recrutar microrganismos benéficos no solo, devido a regulação enzimática. Tais estudos demonstraram um aumento de abundância na rizosfera das plantas dos filos Cyanobacteria, Actinobacteria, Firmicutes, Verrucomicrobiota, Acidobacteria, Bdellovibrionota, Myxococcota, Gemmatimonadota e Planctomycetota, associados ao metabolismo de nitrogênio e enxofre (Nivetha et al. 2024). Os polissacarídeos presentes no extrato algal podem servir como fontes de carbono para grupos microbianos específicos, enquanto outros compostos podem estimular a atividade microbiana por meio de efeitos de sinalização (Yasmeen et al. 2025). Essas mudanças na comunidade microbiana poderiam contribuir para o ciclo aprimorado de nutrientes, supressão de patógenos e modificação da estrutura do solo, criando benefícios indiretos adicionais ao crescimento e saúde das plantas (Pandey & Saharan, 2025).

4.3.Cevada (*Hordeum vulgare* L.)

A cevada (*H. vulgare*) é um dos cereais mais antigos e estrategicamente importantes cultivados no mundo, destacando-se por sua versatilidade de uso na alimentação animal e na indústria cervejeira. De acordo com o USDA Foreign Agricultural Service (2025), a produção global da cultura na safra 2024/25 está estimada em aproximadamente 143,39 milhões de toneladas, com projeção de crescimento para 153,72 milhões de toneladas em 2025/26 — um incremento de cerca de 7% impulsionado pela recuperação de safras em regiões como a União Europeia (50–56 milhões de t) e Rússia (18–19 milhões de t).

No Brasil, embora a produção seja mais modesta, o cenário é de expansão acelerada, especialmente na região Sul, de clima subtropical e temperado. Segundo o 8º Levantamento da Safra de Grãos da CONAB (2025), a safra 2023/24 totalizou 438,4 mil toneladas em 123,1 mil hectares, com produtividade média de 3.561 kg/ha. Para a safra 2024/25, a estimativa é de 510,2 mil toneladas — um aumento expressivo de 16,4%. A produção nacional concentra-se no Paraná (cerca de 80%), seguido pelo Rio Grande do Sul e Santa Catarina, com avanços recentes em São Paulo e potencial de expansão para o Cerrado via sistemas irrigados. Esse crescimento é sustentado por investimentos em cultivares adaptadas, como variedades malteiras de ciclo curto, e por parcerias

estratégicas entre cooperativas e indústrias como a Ambev, visando reduzir a dependência externa — o Brasil ainda importa cerca de 922 mil toneladas de cevada anualmente.

Economicamente, a cevada exerce papel central na cadeia cervejeira global. Estima-se que 25–30% da produção mundial seja destinada à maltaria, abastecendo um mercado que movimenta bilhões de dólares. O Brasil, como terceiro maior produtor mundial de cerveja (~15 bilhões de litros/ano), demanda cerca de 1,93 milhão de toneladas de malte, mas produz internamente apenas 955 mil toneladas, o que equivale a cerca de metade das necessidades. Esse déficit tem estimulado investimentos como a Maltaria Campos Gerais, com capacidade projetada de 240 mil t/ano. No entanto, desafios como flutuações climáticas afetam diretamente a qualidade do grão para maltaria, cujo teor de proteína deve ser inferior a 12%, e eventos extremos, como secas severas, podem reduzir o rendimento em até 30% em regiões marginais, evidenciando a vulnerabilidade do setor a choques de oferta.

Do ponto de vista ecofisiológico, a cevada é uma espécie diploide, de fotoperíodo sensível e ciclo anual, que apresenta relativa tolerância a estresses abióticos quando comparada a outros cereais. Essa resiliência deve-se a mecanismos como sistema radicular profundo para extração de água, ajuste osmótico mediado por acúmulo de prolina e outros solutos compatíveis, e regulação estomática dependente de ácido abscísico (ABA), que preserva o conteúdo relativo de água e a atividade fotossintética sob déficit hídrico moderado. No entanto, as mudanças climáticas têm intensificado a ocorrência de estresses combinados, como déficit hídrico terminal durante o espigamento e ondas de calor (>30–35°C) na antese, que podem reduzir a produtividade em até 50% por abortamento floral, menor número de grãos por espiga e redução no peso de mil grãos (Elakhdar et al., 2022). Genótipos tolerantes, como linhagens selvagens ou melhoradas (ex.: 'Otis'), apresentam maior estabilidade produtiva sob estresses múltiplos (Abdelghany et al., 2024). No contexto brasileiro, onde a cultura se concentra em regiões sujeitas a veranicos e secas prolongadas (cenários agravados pelas projeções do IPCC), o manejo integrado torna-se essencial. Estratégias como seleção de cultivares precoces (escape térmico), plantio direto para conservação de umidade, uso de bioestimulantes e reguladores de crescimento, além de ferramentas de edição gênica (CRISPR) para fortalecer vias de tolerância, são alternativas promissoras para mitigar perdas projetadas de 10–20% na produtividade global até 2050.

4.3.1. Cevada como Modelo para a Validação de Bioinsumos

A cevada tem se consolidado como um modelo vegetal relevante para a validação de bioestimulantes à base de extratos de algas marinhas, com estudos demonstrando melhorias significativas na germinação, no crescimento e na produtividade. Trabalhos seminais, como o de Rayorath et al. (2008), revelaram que extratos da alga parda *Ascophyllum nodosum* podem induzir a atividade da amilase de forma independente do ácido giberélico (GA_3) em sementes de cevada, promovendo um vigor germinativo superior. Em continuidade, Goñi et al. (2021) demonstraram, em ensaios de campo plurianuais, que um bioestimulante derivado de *A. nodosum* aumentou a eficiência do uso de nitrogênio (NUE) em até 60,26%, permitindo a manutenção dos rendimentos mesmo com uma redução de 25% na adubação nitrogenada. De forma análoga, a aplicação de extrato de *Ecklonia maxima* em cevada cervejeira resultou em um incremento de 17% na produtividade e de 17,9% na NUE, possibilitando uma redução significativa no aporte de fertilizantes sem comprometer a qualidade dos grãos para a maltaria (Cozzolino et al., 2021). Mais recentemente, Babazadeh et al. (2023) demonstraram que o extrato da alga parda *Sargassum angustifolium* foi capaz de mitigar os danos causados pelo estresse por frio em plantas de cevada, melhorando parâmetros fotossintéticos e reduzindo o dano foliar sob temperaturas de congelamento. Esses achados, em conjunto, validam a cevada como uma cultura responsiva a bioestimulantes de diferentes espécies de algas marinhas, abrindo um precedente científico à investigação de novos extratos algais. Nesse contexto, a validação do efeito bioestimulante do extrato de *K. alvarezii* em cevada, uma matéria-prima estratégica para o setor cervejeiro nacional, é não apenas oportuna, mas necessária.

5. Formatação da Tese

A tese está estruturada em quatro capítulos, cada um correspondente a um artigo científico. O Capítulo 1 foi formatado segundo as normas da revista ACS Agricultural Science & Technology (fator de impacto 2,9) e submetido a este periódico. O Capítulo 2, igualmente submetido, seguiu as diretrizes do Journal of Food Biochemistry (fator de impacto 4,2). O Capítulo 3 foi redigido conforme as normas da Algal Research (fator de impacto 5,0) e submetido a esse mesmo periódico. O Capítulo 4 foi publicado no Journal of Applied Phycology (fator de impacto 3,3; DOI: https://doi.org/10.1007/s10811-026-03883-z).

Capítulo 1 – Caracterização físico-química e bioquímica do extrato aquoso de *Kappaphycus alvarezii*

Seasonal Variability Shapes Biochemical Composition of a *Kappaphycus alvarezii* Biostimulant Produced in Subtropical Southern Brazil

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Abstract

Biostimulants derived from marine seaweeds are recognized for their economic and environmental benefits, with promising applications across diverse crops. Although Brazil has an extensive coastline, its commercial seaweed production remains quite below its potential. This study aims to biochemically characterize the aqueous extract with claimed biostimulant properties of the red seaweed *Kappaphycus alvarezii* grown in Southern Brazil. The biochemical analyses included total carbohydrates (TC), total soluble sugars (TSS), total starch (TS), total phenolic content (TPC), total proteins (TP), total amino acids (TAA), and macro- and micronutrients. Results showed TC ranging from 0.16 to 8.84 mg/g, TSS from 0.33 to 17.39 mg/g, TS from 1.08 to 18.37 mg/g, TPC from 2.22 to 23.17 mg/100g, TP from 5.38 to 50.63 mg/100g, and TAA from 0.52 to 6.90 mg/g. Potassium was the most abundant macronutrient, ranging from 0.97% to 1.82%. Principal Component Analysis (PCA) revealed clear distinctions in the biochemical and nutrient composition between summer- and winter-collected samples. Significant negative correlations were found between sea surface temperature (°C) and the contents of TAA, TS, TC, TSS, and TPC, as well as the nutrients P, K, S, and Zn. These findings reveal that key nutrients and bioactive compounds in *K. alvarezii* biostimulants accumulate more effectively during colder periods, likely due to the lower temperatures found in the subtropical region where that macroalga species has been grown in Southern Brazil. Understanding these seasonal biochemical patterns is essential for optimizing the cultivation and consistency of seaweed-derived biostimulants for agricultural applications.

Keywords: Seaweed extract; biofertilizer; marine biotechnology; biochemical characterization; Rhodophyta.

1. Introduction

Demographic growth has led to an increasing demand for food, placing continuous pressure on agricultural production systems. However, balancing high productivity with environmental preservation remains a challenge, driving the search for more sustainable cultivation practices. In this context, green agriculture emerges as a pivotal response to contemporary global challenges, making the demand for practices that promote ecosystem resilience more pressing than ever. Consequently, the use of biological inputs (Sarkar et al., 2020; Molotoks et al., 2021), such as biostimulants, has gained significant prominence (Malik et al., 2020; Kapoore et al., 2021).

The term biostimulant refers to substances of biological origin that enhance plant metabolism, promoting growth, yield, and/or improving tolerance to biotic and abiotic stresses (Yakhin et al., 2016). Several studies have highlighted the contributions of algal extracts, i.e. a complex mixtures of bioactive compounds such as carbohydrates, amino acids, proteins, phytohormones, and osmoprotectants, to crop productivity (Ali et al., 2021; Munaro et al., 2021).

Red seaweeds (Rhodophyta), with around 6,000 species, are rich in minerals, vitamins, and secondary metabolites, making them valuable raw materials for various industries (Carpena et al., 2022). *Kappaphycus alvarezii* (Doty) Doty ex P.C.Silva, 1996, shows significant potential as a biostimulant (Ali et al., 2021), enhancing crop productivity in sugarcane (Karthikeyan and Shanmugam, 2017; Singh et al., 2018), maize (Layek et al., 2015; Basavaraja et al., 2018), pepper (Melo et al., 2020), and cucumber (Shukla et al., 2023). In wheat, it increased nutrient absorption and yield (Shah et al., 2013), improved drought resistance (Patel et al., 2018) and boosted rice germination and seedling vigor (Layek et al., 2018). Additionally, *K. alvarezii*'s biostimulant can reduce CO_2 emissions in sugarcane (Singh et al., 2018) and enhance soil microbiota in maize (Nivetha et al., 2024), making those crops systems less impactful to the environment.

K. alvarezii cultivation began in the tropical coastal regions of Southeast Asia, notably in the Philippines, Indonesia, and Malaysia. Consequently, the biochemical profile of its aqueous extract with plant biostimulant properties largely reflects the climatic conditions of those sites, as well as the cultivation practices adopted (Kumar et al., 2020a; Vaghela et al., 2022; 2023a). With the recent approval for large scale

cultivation of *K. alvarezii* in marine farms in Brazil (IBAMA – Brasil, 2020), there is now an urgent need for comprehensive biochemical analysis of locally produced and already commercialized biostimulants of this seaweed species. This is particularly important given the recognized significance of regional variations in the chemical properties of seaweeds (Demes and Pruitt, 2019) and their derivative products. Moreover, studies have documented seasonal changes in the chemical composition and nutritive value of this seaweed in established cultivation areas (Deyab, 2011; Khairy and El-Shafay, 2013; Afonso et al., 2021; Vázquez-Delfín et al., 2023; Araújo et al., 2022; Schneider et al., 2026). Understanding these variations is essential to ensure that the seaweed-based biostimulant meets regulatory standards and is optimized for its intended applications in crop systems.

Santa Catarina State, located in southern Brazil (subtropical climate zone), has emerged as a strategic hub for *Kappaphycus alvarezii* cultivation and biostimulant production. In 2024, the state produced 751 tons of fresh seaweed biomass and 600,872 liters of biostimulant, generating approximately USD 1,991,840 in revenue from biostimulant sales alone (Santos, 2024). Although commercial-scale cultivation of this red seaweed remains in its nascent stages, these results underscore southern Brazil's considerable potential as a viable region for both *K. alvarezii* aquaculture and the valorization of its by-products (Hayashi et al., 2024).

Thus, this work aimed to biochemically characterize the *K. alvarezii*-based biostimulants produced over the 2022-2023 growth season in two marine farms in Santa Catarina State (southern Brazil). Unless stated otherwise**,** this is the first detailed biochemical assessment of this red algae species derived from commercial cultivation in the subtropical southern Atlantic. Comprehensive biochemical analyses were conducted to ensure that the algal biostimulant complies with regulatory standards and is optimized for its intended applications in agricultural systems.

2. Material and Methods

2.1. Sample and sampling sites characterization

The samples of *K. alvarezii* extracts were kindly furnished by two marine seaweeds farm companies located in the Ribeirão da Ilha beach (Florianópolis city, RIB

- 27° 42' 32.724" S, 48° 33' 35.5" W) and Enseada do Brito beach (Palhoça city, PAL - 27° 46' 29.928" S, 48° 37' 50.7" W), Santa Catarina state (southern Brazil - Fig. 1).

Both sampling sites are located in a subtropical zone and has a humid oceanic climate with no dry season and hot summers (Cfa, according to Koppen's classification). The Cfa climate type comprises a maximum average temperature over 22ºC and a minimum average between -3 and 18ºC. The rainfall is well distributed throughout the year (Alvares et al., 2013), with annual accumulated precipitation of 2016 mm in 2023 in the Santa Catarina Island (INMET, 2024).

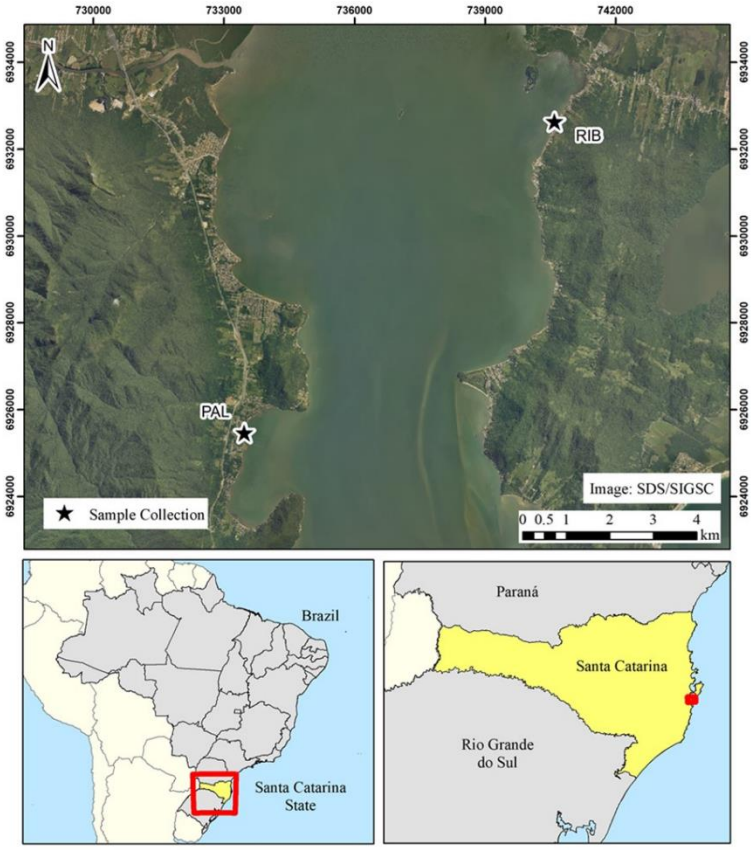

Fig. 1. The sampling sites of *Kappaphycus alvarezii* marine farms: Palhoça (PAL – continent side), and Florianópolis (RIB – island side), south bay of Santa Catarina Island, state of Santa Catarina, Southern Brazil (Nunes et al., 2024b).

Due to seasonal variations in environmental conditions, which could influence the physiology and biochemical composition of seaweeds, biostimulant samplings were carried out seasonally from April 2022 to March 2023 (Table 1). The samplings were

concentrated in the last two-thirds of the seasons, allowing the analyses to reflect the environmental characteristics of each season.

Table 1. Season, Site of Production, ID, and date of *Kappaphycus alvarezii* sampling, and monthly average of the sea surface temperature (SST, °C) until the sampling date.

PAL – Palhoça; RIB – Florianópolis.

Season	Site of Production	ID	Date	SST (°C)
Autumn	PAL	PAL-1	04/26/2022	22.44 (± 0.39)
		PAL-2	06/14/2022	17.02 (± 0.41)
	RIB	RIB-1	04/19/2022	26.05 (± 0.41)
		RIB-2	06/07/2022	22.96 (± 0.40)
Winter	PAL	PAL-3	07/26/2022	17.60 (± 0.39)
		PAL-4	09/20/2022	17.97 (± 0.40)
	RIB	RIB-3	07/19/2022	17.63 (± 0.38)
		RIB-4	09/13/2022	17.93 (± 0.39)
Spring	PAL	PAL-5	11/08/2022	19.67 (± 0.38)
		PAL-6	12/13/2022	22.05 (± 0.40)
	RIB	RIB-5	10/25/2022	19.07 (± 0.40)
		RIB-6	12/06/2022	22.78 (± 0.40)
Summer	PAL	PAL-7	02/07/2023	25.45 (± 0.40)
		PAL-8	03/14/2023	26.05 (± 0.41)
	RIB	RIB-7	01/24/2023	26.16 (± 0.40)
		RIB-8	03/07/2023	25.41 (± 0.40)

The production of the seaweed biostimulant samples was made directly by the companies. In this process, the seaweeds were initially washed with seawater to eliminate impurities and fouling organisms, then chopped using an industrial blender. The resulting biomass was then mechanically pressed, and the extracted sap was filtered, yielding about 800 mL/kg fresh alga biomass. Approximately 5 L samples were transferred to clean plastic containers and transferred to the laboratory. At the laboratory, the samples were aliquoted into 50 mL falcon tubes and frozen at -20°C for subsequent biochemical analysis. Before freezing, an aliquot of the samples was analyzed for total solid content, pH, and electrical conductivity.

2.2 Total Solid Content (TSC), pH and electrical conductivity (Cond)

For the determination of the total solid content (TSC), a gravimetric method was used. For that, 10 g of each biostimulant sample were transferred to plastic petri dishes, frozen (-80°C), and freeze-dried (~20 h, -55°C, 0.030 mbar). The solid content values were obtained by the rate between the dried and liquid weights, using the following formula:

$$TSC = \frac{Dw}{Aw} \times 100$$

Where: TSC = total solid content, Dw = dried weight, and Aw = aqueous weight (10 g). The pH was measured with a pHmeter (Sensoglass - pHmeter SP1800, São Paulo - Brazil), previously calibrated, while conductivity (Cond – mS/cm) was measured with a conductivity meter (HM Digital - COM-80, China). To determine the pH and Cond values, samples were previously diluted ten times and measured in triplicate.

2.3 Total carbohydrate (TC) content

The TC content was determined as described by Dubois et al. (1956), with adaptations. Briefly, 50 µL each sample was diluted in 20 mL distilled water and centrifuged at 4000 rpm, for 10 min. Then, 2 mL of the supernatant were transferred to a test tube and mixed with 0.05 mL of 80% phenol and 5 mL of concentrated sulfuric acid. After 10 min, the samples were vortexed and incubated in a water bath (30°C, 15 min). Finally, the absorbance of the reaction medium (n = 3) was read at 490 nm, using a microplate reader (SpectraMax 190 Microplat Reader, Molecular Devices, California, USA). Galactose (99%, Sigma-Aldrich, MO, USA) was used to generate a standard curve (7.8-500 µg/mL; $y = 0.0025x$, $r^2 = 0.9992$). The TC contents measured were converted from µg/mL to mg/g for further analysis.

2.4 Total soluble sugars (TSS) concentration

The TSS concentration was analyzed in accordance with Umbreit and Burris (1964) with adaptations. For that, the biostimulant sample (50 µL) was added of 2 mL

methanol: chloroform: water solution (M:C:W, 12:5:3, v/v/v) and centrifuged (4000 rpm, 10 min). The supernatant was collected and the extraction repeated on the remaining pellet. The combined supernatants were made up to 4 mL with M:C:W solution. For the reaction, 1 mL chloroform and 1.5 mL distilled water were added to the extract, following the recovery of the upper aqueous phase. Then, 2 mL of 0.2% anthrone (Sigma-Aldrich) were added and the mixture heated at 100°C, for 3 min. After cooling, the absorbance was read at 630 nm using a UV-vis spectrophotometer (SpectraMax 190 Microplat Reader, Molecular Devices, California, USA). For purpose of calculation of TSS contents, a standard curve of glucose (99%, Sigma-Aldrich, MO, USA) was build (62.5-2000 µg/mL; $y = 0.0018x$, $r^2 = 0.9517$). The TSS measures (n = 3) were further converted from µg/mL to mg/g.

2.5 Total starch (TS) content

The TS amount was determined following the Umbreit and Burris (1964) method. For that, 2 mL 30% perchloric acid were added to the residual pellet from the TSS extraction, centrifuged (4000 rpm, 10 min) and the supernatant was collected. This extraction was repeated and the combined phases were used for the TS analysis. After, 1 mL of the extract was mixed with 2 mL 0.2% anthrone (Sigma-Aldrich, Sao Paulo, Brazil) solution, heated at 100°C for 3 min, and then cooled. The absorbance was recorded at 630 nm using a microplate reader (SpectraMax 190 Microplat Reader, Molecular Devices, California, USA). Total starch content was calculated using a standard curve (62.5-2000.0 µg/mL; $y = 0.0016x$, $r^2 = 0.9916$) built with an analytical standard of starch (98%, Sigma-Aldrich, Sao Paulo, Brazil). The TS values were determined (n = 3) and further converted from µg/mL to mg/g.

2.6 Total phenolic content (TPC)

The TPC was analyzed following the method proposed by Singleton and Rossi (1965), with adaptations. Briefly, 300 µL of biostimulant sample were added of 3 mL 80% methanol, incubated in the dark for 1 h, and then centrifuged (4,000 rpm, 5 min). To 100 µL of the supernatant, 75 µL Folin-Ciocalteu reagent (2N) and 825 µL 2% (w/v) sodium carbonate solution were added. After 1 h of incubation, 300 µL of the sample was

pipetted into microplates (n = 3), and the absorbance was measured at 750 nm using a microplate reader (SpectraMax 190 Microplat Reader, Molecular Devices, California, USA). Gallic acid (98%, Sigma-Aldrich, Sao Paulo, Brazil) was used to generate a standard curve (7.81-500 μg/mL; $y = 0.006 \times$, $r^2 = 0.9967$). The TPC measured were then converted from μg/mL to mg/100 g.

2.7 Total protein (TP) content

The content of TP in the biostimulant samples was analyzed following the Bradford (1976) method. For that, 100 μL of the sample were mixed with equal volume of phosphate-buffered saline (PBS, pH 7.4; Sigma-Aldrich, MO, USA). Then, 5 mL of a solution containing the Bradford reagent and water (1:5, v/v) was added to the mixture and incubated for 5 min. The sample absorbance (n = 3) was measured at 595 nm using a microplate reader (SpectraMax 190 Microplat Reader, Molecular Devices, California, USA). Bovine serum albumin (Sigma-Aldrich, Sao Paulo, Brazil) was used to generate a standard curve (0.98-62.50 μg/mL; $y = 0.0032x$, $r^2 = 0.9882$). The TP amounts measured were further converted from μg/mL to mg/100 g.

2.8 Total amino acids (TAA) concentration

Previously to the TAA content analysis, the biostimulant samples were hydrolyzed according to the method of Silveira and Furlong (2007), with adaptations. The samples were diluted in distilled water (1: 30) and acidified with HCl (0.01 M) until pH ~3. Afterwards, 1 mL diluted sample was mixed with 3 mL 0.2% (w/v) ninhydrin solution (Sigma-Aldrich, MO, USA) prepared in 200 mM sodium phosphate buffer (pH 7.0). The samples were incubated in a water bath at 100°C for 30 min, following the recording of the absorbance (n = 3) at 570 nm in a microplate reader (SpectraMax 190 Microplat Reader, Molecular Devices, California, USA). Glutamic acid (99%, Sigma-Aldrich, Sao Paulo, Brazil) was used to generate a standard curve (0-150 μg/mL; $y = 0.0028x$, $r^2 = 0.9932$). The TAA contents determined were then converted from μg/mL to mg/g.

2.9 Heavy metals, macro- and micronutrients

The analyses of heavy metals, macro- and micronutrients were performed by the Fundação ABC company (Brazil) using the EPA protocols 3051A (2007) and 6010D (2018), respectively. For these analyses the samples were grouped by season. Macronutrients such as Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, and Sulphur were analyzed and expressed as percentages, while micronutrients including Boron, Copper, Iron, Manganese, Sodium, and Zinc were measured in mg/kg. The total organic Carbon in the aqueous extracts was also examined.

2.10 Statistical analysis

Data are expressed as means ± standard deviation (SD, n = 3) and were compared by ANOVA using the four seasons (Autumn, Winter, Spring, vs Summer) and two sites of production (PAL vs. RIB) as fixed and independent factors, with scripts written in R (version 4.0.2). The biochemical variables analyzed included TSC, pH, Cond, TC, TSS, TS, TPC, and TAA. To ensure that the assumptions of normality were met before applying ANOVA, the variables were transformed using the *bestNormalize* package (Peterson, 2021) in R software.

Subsequently, principal component analysis (PCA) was performed using the singular value decomposition (SVD) algorithm, following smoothing with a three-point Gaussian filter. Further, a heatmap analysis was applied to the macro- and micronutrients, as well as to the organic carbon data set, followed by PCA using the SVD algorithm in The Unscrambler® X (Version 10.4).

To corroborate the influence of the seasons on the biochemical and nutrient composition of the biostimulants, the sea surface temperature (SST) data set was also considered as an additional factor. The SST data was obtained from the Multiscale Ultrahigh Resolution (MUR) Level 4 analysis, made available by GHRSST (Group for High Resolution Sea Surface Temperature) and processed at the JPL Physical Oceanography DAAC, with a spatial resolution of 0.01 degree. Table 1 shows the thirty-day mean of SST of each site where seaweed samples were harvested (more detailed climate information in supplementary material – Fig S1). To verify the correlation between SST and all biochemical and nutrient contents, a multivariate Spearman

correlation analysis was performed using the *corrplot* package (version 0.92) in R language (Wei, 2021).

3. Results

The growing demand for sustainable agricultural biostimulants has led to increased interest in seaweeds, which can be cultivated on a large scale and are rich sources of macro- and micronutrients, as well as bioactive compounds beneficial to various crop species. With its extensive coastline, Brazil holds significant potential to become a major global producer of seaweed. In this study, the biochemical profiles of the first commercial *K. alvarezii* biostimulant produced in two marine farms in southern Brazil were determined. For that, samples were seasonally collected and investigated over one year (April 2022 to March 2023). All results are represented in the following sections, but more detailed data can be found in the supplementary material.

3.1. Total Solid Content (TSC), pH, and electrical conductivity (Cond)

The initial analysis of the *K. alvarezii* biostimulant covered total solid content (TSC), pH, and conductivity (Cond), revealing seasonal stability and some distinct trends. TSC values ranged from 2.59% to 5.55%, without significant effects for the factors season and sites of production ($F = 0.989$, $p = 0.408$; $F = 1.721$, $p = 0.638$) (Figure 2A). In their turn, the biostimulant's pH varied from 5.23 ± 0.01 to 7.35 ± 0.05, with a noticeable pH decrease in summer samples (mean $= 5.67 \pm 0.69$) compared to other seasons ($F = 4.603$, $p = 0.007$) (Figure 2B). Electrical conductivity analysis showed no significant seasonal fluctuations ($F = 1.027$, $p = 0.391$), with mean values of 56.72 ± 13.10 mS/cm in autumn, 50.78 ± 18.73 mS/cm in winter, 58.04 ± 9.54 mS/cm in spring, and 51.13 ± 6.90 mS/cm in summer. However, regarding the sites of production of the biostimulant, the PAL-harvested samples exhibited significantly higher electrical conductivity in comparison to the RIB ones ($F = 26.995$, $p < 0.001$), except when collected in autumn (Figure 2C).

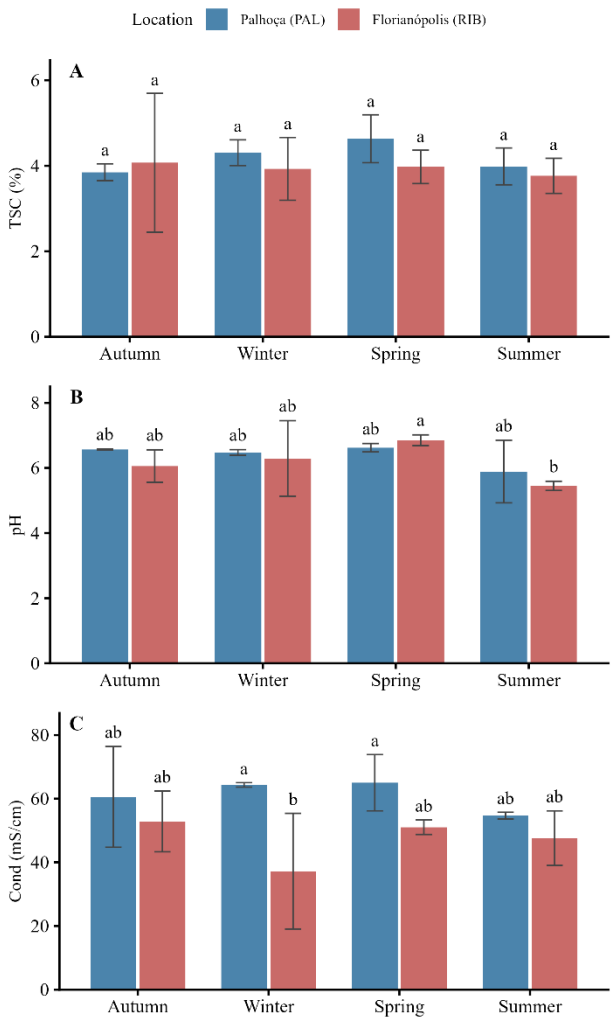

Fig. 2. Seasonal variation of the Total Solid Content (TSC, A), pH (B), and electrical conductivity (Cond, C) of the biostimulant samples of *Kappaphycus alvarezii* cultivated in Palhoça (PAL) and Florianópolis (RIB), Southern Brazil.

3.2. Total Carbohydrates (TC), Total Soluble Sugars (TSS), and Total Starch (TS)

The analyses of the TC, TSS, and TS contents in the *K. alvarezii* biostimulant samples revealed significant seasonal and site-related variations. TC amounts varied widely across seasons and sites of production, with significant main effects for both, as well as an interaction between those factors ($F = 7.258$, $p < 0.001$) (Figure 3A). Specifically, the highest TC concentrations were observed in spring-harvested samples from PAL (7.60 ± 0.96 mg/g), while the lowest value was detected in summer-collected samples from RIB (0.37 ± 0.24 mg/g). TSS contents also revealed statistically significant

differences between samples for both seasonality and production sites, besides the interaction between those factors (F = 2.859, p = 0.049). The samples RIB-2 (autumn, 17.39 ± 0.36 mg/g) and PAL-4 (spring, 14.18 ± 0.43 mg/g) presented superior TSS amounts, contrasting with the lowest mean noted in the summer-produced biostimulant (RIB, 0.34 ± 0.07 mg/g). Interestingly, the seasonal averages were detected for that variable in the colder harvest seasons, i.e., 5.33 ± 7.33 mg/g in autumn and 4.82 ± 5.69 mg/g in winter, in respect to the warmer ones, 2.53 ± 1.37 mg/g – spring, and 0.68 ± 1.68 mg/g - summer (Figure 3B). For TS concentrations, samples differed only over the production seasons, with summer samples showing the lowest amounts compared to the other seasons (F = 12.478, p < 0.001), as also noted for the TC and TSS contents. The mean TS amounts over the seasons showed the highest value in autumn (i.e., 7.86 ± 6.57 mg/g) in respect to the other seasons, e.g., 5.68 ± 2.89 mg/g - winter, 5.72 ± 1.61 mg/g - spring, and 1.80 ± 0.53 mg/g in summer (Figure 3C).

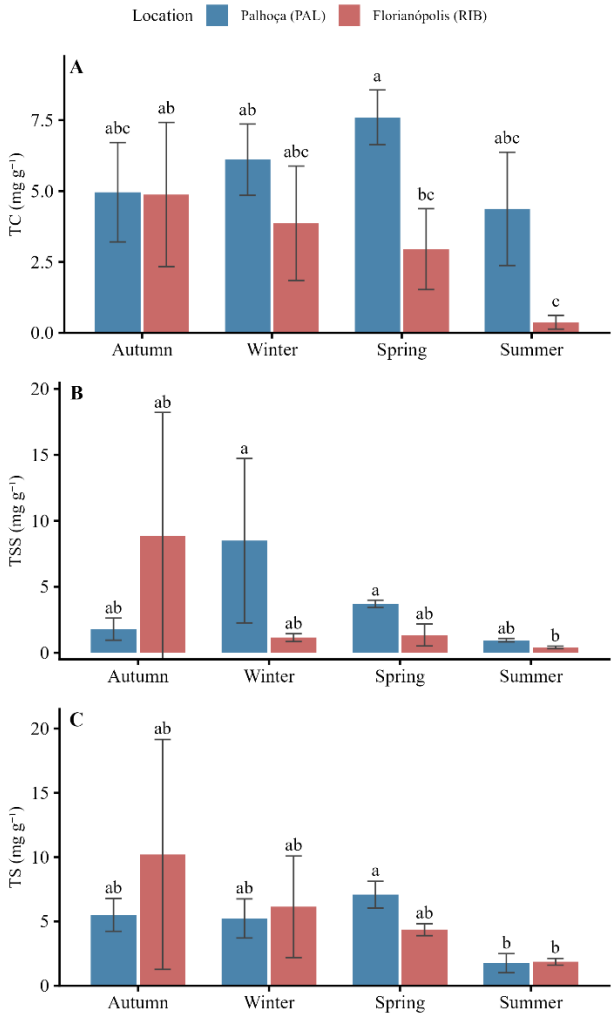

Fig. 3. Seasonal variation of the Total Carbohydrates (TC, A), Total Soluble Sugars (TSS, B), and Total Starch (TS, C) of biostimulant samples of *Kappaphycus alvarezii* cultivated in Palhoça (PAL) and Florianópolis (RIB), Southern Brazil.

3.3. Total Phenolic Content (TPC), Total Protein (TP), and Total Amino Acids (TAA)

For TPC, significant differences were detected between the biostimulant samples collected across the seasons ($F = 8.275$, $p < 0.001$) and production sites ($F = 15.433$, $p < 0.001$), with no interaction effects. The highest TPC concentration was found in autumn samples from PAL (22.33 ± 1.90 mg/100 g), while the lowest one was detected in summer samples from the same site (1.17 ± 0.15 mg/100 g). Seasonal averages of TPC were 11.33 ± 7.13 mg/100 g (autumn), 15.04 ± 3.42 mg/100 g (winter), 11.64 ± 7.99 mg/100 g (spring), and 5.71 ± 4.86 mg/100 g (summer - Figure 4A).

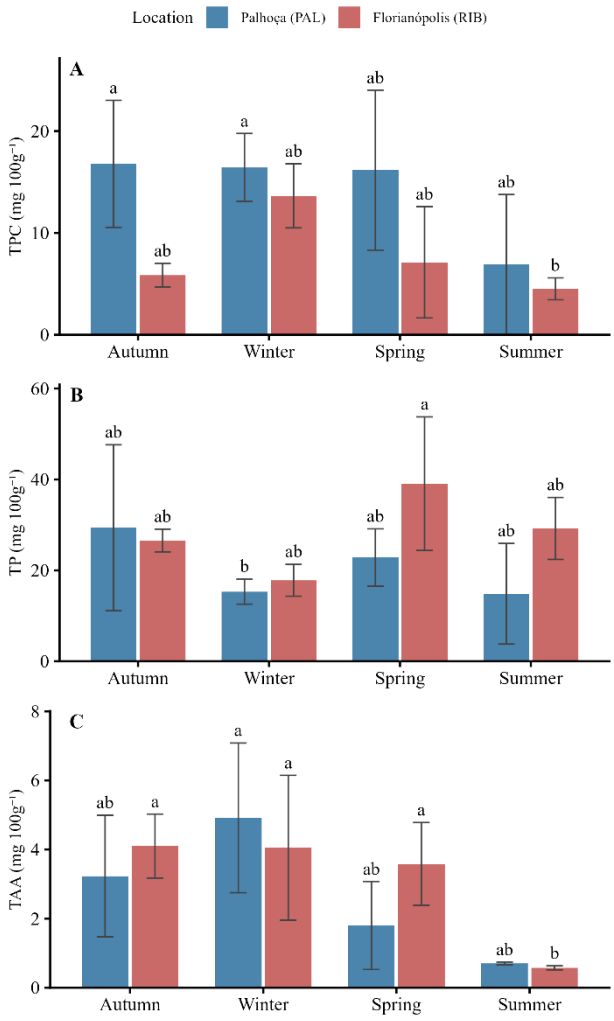

Fig. 4. Seasonal variation of the Total Phenolic Content (TPC, A), Total Proteins (TP, B), and Total Amino Acids (TAA, C) of the biostimulant samples of *Kappaphycus alvarezii* cultivated in Palhoça (PAL) and Florianópolis (RIB), Southern Brazil.

The average values of TP varied significantly across the seasons, with the highest concentration recorded in spring samples from RIB (50.63 ± 11.41 mg/100 g) and the lowest one in summer samples from PAL (5.31 ± 1.43 mg/100 g). The overall winter samples exhibited the lowest average values (16.57 ± 3.28 mg/100 g), which differed significantly from the other seasons ($F = 5.121$, $p = 0.004$). The average TP values for the remaining seasons were 27.97 ± 12.50 mg/100 g in autumn and 30.94 ± 13.72 mg/100 g in spring (Figure 4B). For the TAA contents, the highest amount was detected in winter-collected samples from PAL (6.90 ± 0.01 mg/100 g), as RIB samples showed superior concentrations in autumn (5.97 ± 0.01 mg/100 g). Interestingly, the lowest

concentrations of those metabolites were observed in summer for both sites (0.52 - 0.74 ± 0.01 mg/100 g). Seasonal averages for TAA were 3.67 ± 1.41 mg/100 g (autumn), 4.48 ± 2.08 mg/100 g (winter), 2.69 ± 1.50 mg/100 g (spring), and 0.64 ± 1.64 mg/100 g (summer - Figure 3C), ($F = 4.503$, $p < 0.001$).

3.4. Heavy Metals, Macro- and Micronutrients

Heavy metals analysis revealed no detectable levels in the *K. alvarezii* biostimulant samples. Additionally, to analyze the potential of *K. alvarezii* as a source of readily available nutrients for plants, it is important to identify the mineral components present in its aqueous extract. The values of macro- and micronutrients and total organic carbon content found in the extracts studied are summarized in the heatmap (Table 2). Potassium (K) was the most abundant element, ranging from 0.97% to 1.82%. The higher K values were detected in samples collected in the late autumn period (PAL-2 and RIB-2 samples), while the lowest amounts were found both in late spring and summer samples (PAL and RIB 6, 7, and 8) (Table 2). By comparing the samples according to their sites of production, all PAL samples showed higher K levels than the RIB ones, regardless of the collection season (t-test $p = 0.028$). The organic carbon (C) was the second most abundant component present in the *K. alvarezii* extracts, but with a wide range of contents, i.e., from 0.04% to 1.87%. However, a trend of increasing concentrations during winter and decreasing during summer can be observed, particularly in PAL samples, not being evident in RIB ones (Table 2). The amounts of the other macronutrients also varied according to season and the sites of production investigated, e.g., phosphorus (0.006% - 0.011%), calcium (0.007% - 0.018%), and sulfur (0.023%-0.098%) (Table 2).

Table 2. Heatmap of organic carbon, macronutrients, and micronutrients concentrations in biostimulant samples extracted from the seaweed *Kappaphycus alvarezii* cultivated in Palhoça (PAL) and Florianópolis (RIB), Southern Brazil, in 2022-2023.

3.5. Principal component analysis (PCA) and multivariate Pearson correlation analysis

The PCA was applied to the biochemical dataset (i.e., TC, TSS, TS, TPC, TP, and TAA) of the biostimulant samples. The total variance of data captured by the PC1, PC2, and PC3 latent variables summed up 85%, with PC1 representing 46%, PC2 representing 21% (Fig. 5), and PC3 17% (supplementary material – Fig. S2). PCA was able to identify and discriminate two distinct groups regarding the seasonal effect on the *K. alvarezii*'s biochemical profiles, i.e., summer- and winter-harvested samples. The former samples grouped in the PC1-, while the winter ones (except RIB-4) grouped in the PC1+ quadrant (Fig. 5). Furthermore, the loading analysis showed that PAL-2 and PAL-4 samples were correlated with TC, TSS, and TS concentrations, while PAL-3, RIB-3, and PAL-5 grouped according to their TAA amounts and PAL-1 and RIB-5 presented similar PT contents (Fig 5).

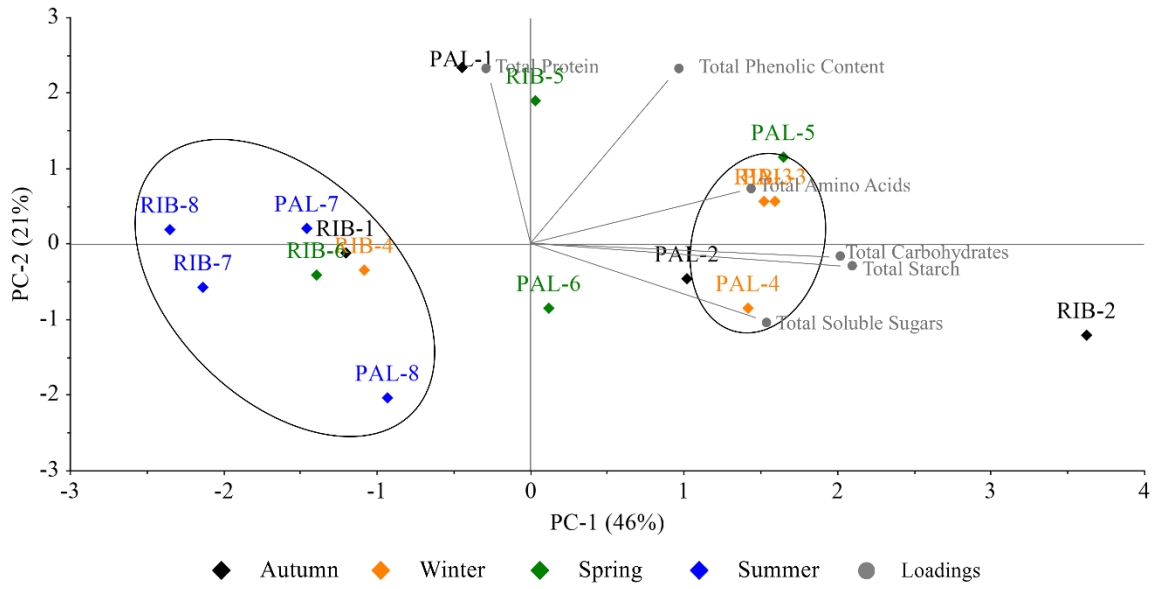

Fig. 5. Score plot of principal component analysis (PCA) of the biochemical profile dataset of the biostimulant samples extracted from *Kappaphycus alvarezii* cultivated during autumn, winter, spring, and summer in Palhoça (PAL) and Florianópolis (RIB), Southern Brazil.

The descriptive model resulting from the PCA for the macro- and micronutrients computed a total variance of 77% for the dataset, with PC1 accounting for 49%, PC2 15% (Fig. 6), and PC3 13% (supplementary material – Fig. S3). Similar to the results found for the biochemical profiles, a clear discrimination of samples between the two sites (i.e., PAL and RIB) was not detected, with the samples grouping primarily by seasonality. The samples were

grouped by the beginning and end of the seasons, with PAL and RIB samples clustering together. This pattern was observed for samples PAL 1 and RIB 1 (PC2+ and PC2-; beginning of autumn), PAL 2 and RIB 2 (PC1+; end of autumn), PAL 7 and RIB 7 (PC2-; beginning of summer) and PAL 8 and RIB 8 (PC2+; end of summer). The winter samples were found closer to each other (PC1+ and PC2-), as well as three spring samples (PC1+). The loading analysis showed that RIB-3 and RIB-4 samples have strong correlations with P, K, S, Ca, and Zn amounts, while PAL-3 was correlated with Cu contents, and PAL-3 and RIB-4 with the concentrations of Mn and Fe. Additionally, samples PAL-2 and RIB-2 were correlated with Mg, B, and Na, respectively (Fig 6 and 3S).

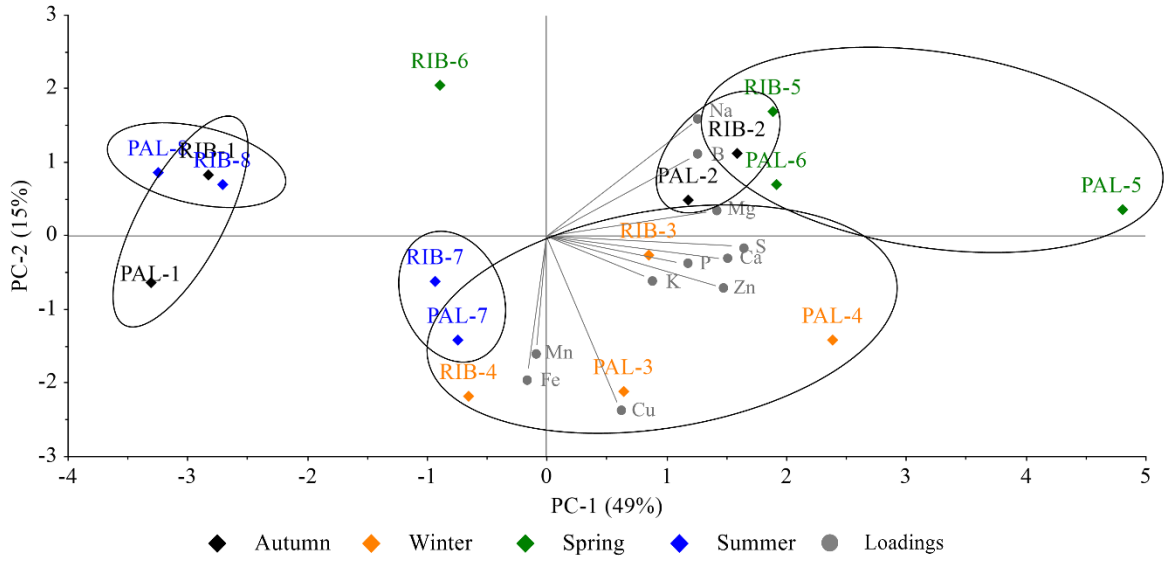

Fig. 6. Score plot and factor loading of principal component analysis (PCA) of the macro- and micronutrient dataset of the biostimulant samples extracted from *Kappaphycus alvarezii* cultivated during autumn, winter, spring, and summer in Palhoça (PAL) and Florianópolis (RIB), Southern Brazil.

To explore the influence of environmental factors such as the seawater surface temperature (SST) on the biochemical profile and nutrient contents of the algal biostimulant samples, a multivariate Spearman correlation analysis was carried out. The analysis revealed significant negative correlations ($p < 0.05$) between SST and TAA (ρ = -0.76), TS (ρ = -0.67), TSS (ρ = -0.62), TPC (ρ = -0.57), and TC (ρ = -0.52). Similarly, certain nutrients exhibited

significant negative correlations with SST, including S (ρ = -0.73), K (ρ = -0.62), P (ρ = -0.60), and Zn (ρ = -0.52) (Fig. 7).

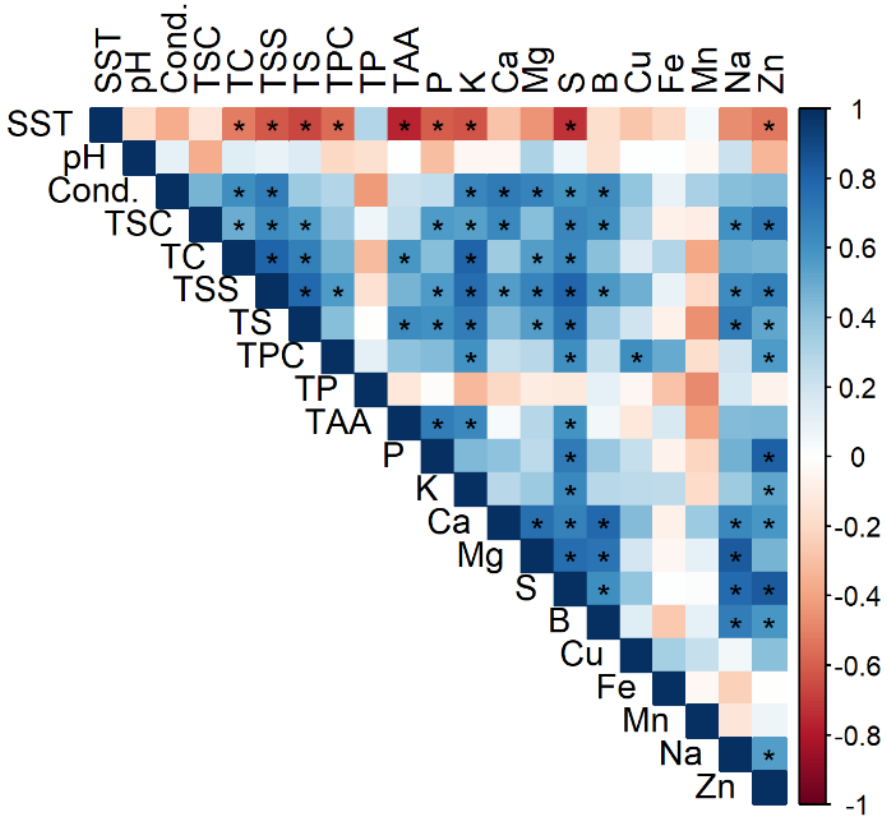

Fig. 7. Spearman correlation coefficients between seawater surface temperature (SST) and biochemical components, macronutrients, and micronutrients present in biostimulants extracted from the seaweed *Kappaphycus alvarezii* cultivated in Palhoça (PAL) and Florianópolis (RIB), Southern Brazil. Biochemical components include pH, electrical conductivity (Cond.), total solid content (TSC), total carbohydrates (TC), total soluble sugars (TSS), total starch (TS), total phenolic content (TPC), total protein (TP), and total amino acids (TAA). Macronutrients comprise Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), and Sulfur (S), while micronutrients include Boron (B), Copper (Cu), Iron (Fe), Manganese (Mn), Sodium (Na), and Zinc (Zn). Squares marked with * indicate statistically significant correlations ($p < 0.05$).

4. Discussion

The biochemical and nutritional profile of *K. alvarezii* biostimulants exhibited significant seasonal and spatial variations, underscoring how environmental conditions

modulate their composition. Seasonal shifts (summer vs. winter) and distinct cultivation sites influenced critical biochemical and nutritional parameters in the biostimulants, reflecting the interplay between algal physiology and localized environmental drivers.

4.1 Biochemical content

Total solid content (TSC), a key indicator of organic compounds like polysaccharides and phenolics in seaweed extracts (Ashour et al., 2022; Vaghela et al., 2022), aligned with values reported for tropical *K. alvarezii* extracts (3–4.47%; Karthikeyan & Shanmugam, 2017; Shanmugam & Seth, 2018). The pH (average 6.28) and electrical conductivity (58.11 mS/cm) further reflect the biostimulant's functional properties. Optimal pH ensures nutrient availability and stabilizes active ingredients (Truog, 1947; Barrow & Hartemink, 2023), while conductivity correlates with dissolved salts, likely influenced by the unprocessed biomass's inherent salinity (Ding et al., 2018; Ferrarezi et al., 2022).

Carbohydrates, e.g., soluble sugars and starch, are important compounds sources of metabolic energy for plant development, serving as substrate for ATP synthesis and metabolic intermediates, for instance, thus influencing cell division and expansion (Eveland and Jackson, 2012). Furthermore, carbohydrates play a crucial role in plant responses to (a)biotic stress factors, enabling plants to withstand conditions, such as high radiation exposure, low temperatures, and hydric deficit (Pommerrenig et al., 2018), as well as the presence of pathogens. The presence of oligo- and polysaccharides in algal extracts (e.g., alginates, fucans, laminarins, and carrageenans) has demonstrated antifungal activity against plant pathogens by activating defense mechanisms in host plants (Agarwal, Dangariya & Agarwal, 2021; Vicente et al., 2021). This induction of defense response is mainly associated with the generation of oxidative bursts and activation of the salicylic acid (SA), jasmonic acid (JA), and ethylene pathways (Vera et al., 2011). Furthermore, the λ-carrageenan was able to induce the activation of defense genes associated to the transcription of relevant enzymatic systems as, for instance, sesquiterpene cyclases, chitinases, and proteinase inhibitors type II in tobacco plants (Mercier et al., 2001). Additionally, sulfated polysaccharide has also induced resistance of *Arabidopsis thaliana* to the fungus *Sclerotinia sclerotiorum* by activating the Jasmonic Acid pathway (Sangha et al., 2010). Moreover, studies have shown that polysaccharides extracted from various seaweed species, including *K. alvarezii*, demonstrate notable antioxidant activities, suggesting their potential application in agricultural practices to increase plant resilience

against environmental stressors (Arunkumar et al., 2021; Bhuyar et al., 2021). Although the antioxidant benefits of carbohydrates are well-documented, further investigation is required to fully understand their mechanisms of action and to optimize extraction methods for practical use in improving plant health and productivity (Liu and Sun, 2020; Bhuyar et al., 2021). Importantly, in addition to carbohydrates, other compounds found in seaweeds, such as phenolic compounds and amino acids, may individually or synergistically contribute to these antioxidant properties (Harb et al., 2021). Thus, such metabolites were also investigated in the samples herein study as further described.

Phenolic compounds are important secondary metabolites that are crucial in physiological roles throughout the plant's life cycle. In addition to influencing growth and development, e.g., seed germination, biomass accumulation, and improved metabolism, the availability of polyphenols can help alleviate plant stress under adverse conditions (Sharma et al., 2019). Therefore, understanding the amount of these compounds in biostimulants is essential for promoting plant maintenance and health (Drobek et al., 2020). When comparing the average values of TPC in the biostimulant samples herein studied (10.93 mg/100 g) with the *K. alvarezii* algal biomass (79.73 mg/100 g – unpublished data), it is possible to note an important reduction, possibly due to the extraction process adopted by industry, which retains a portion of these compounds in the residue. In this sense, the industrial residue from *K. alvarezii* extract production has great potential to be used in biotechnological applications (Nunes et al., 2024a). It is noteworthy that studies characterizing polyphenols in seaweed extracts for agricultural applications are scarce in the literature, as they are predominantly used for medicinal purposes (Cotas et al., 2020). Such a fact was also observed for amino acids and proteins in that algal biomass.

Amino acids, the building blocks of proteins, are essential as they provide important inputs to the plants that allow their growth and development, in addition to playing a role in defense against stresses (Trovato et al., 2021). In general, the concentrations of amino acids and proteins in algal samples fluctuate according to the season and production site. For instance, Araújo et al. (2022) analyzing *K. alvarezii* seaweed biomass samples grown in Brazil found greater mean values of AAs during summer (586.00 to 743.70 μg/g DW), with lower amounts detected in autumn and winter (106.91 to 190.88 μg/g DW), contrary to our findings in the biostimulant samples investigated. Xiren and Aminah (2017) also reported variations in the amino acid content of *K. alvarezii* samples from two distinct sites in Malaysia (Langkawi

Island and the coastal area of Sabah), with values of 3.79 and 5.93 mg/100 g, respectively. Interestingly, these values are comparable to our findings for samples collected during autumn and winter in southern Brazil. Similarly, Hardjani et al. (2017), using raw and fermented *K. alvarezii* extracts, as feed supplement for white shrimp *Litopenaeus vannamei*, identified 8.24% total proteins, which increased to 9.13% after fermentation. Those authors also quantified 16 amino acids, totaling 2.47% pre-fermentation and 3.14% post-fermentation. In studying the chemical composition of industrially used algae biomass, Naseri et al. (2019) quantified 6.62% of total proteins and characterized 16 amino acids. Importantly, the concentration of essential amino acids determines the quality of the proteins found in a given biomass (Damodaran et al., 2008; Adhikari et al., 2022).

It is worth mentioning that while some studies have presented the total amino acid and protein contents from algal extracts, they are often derived from techniques where the chemical or enzymatic hydrolysis of samples is performed, previously to the quantitation of those metabolites (Xiren and Aminah, 2017; Vaghela et al., 2023a). Such an analytical approach increases the content of those compounds and thus their quantification and identification, in comparison to the non-hydrolyzed samples. However, aqueous extracts used as biostimulants, such as in the present study, and others commercially known as K-sap®, do not undergo any hydrolysis process, as they are simply the result of pressing fresh alga biomass (Layek et al., 2015; Patel et al., 2018). Consequently, the content of amino acids and proteins present in *K. alvarezii* aqueous extract does not necessarily reflect those demonstrated by algal extracts in the literature. Therefore, characterizing the content available in aqueous extracts used as biostimulants is essential to verify whether there are free amino acids that can serve as plant growth and/or defense promoters.

4.2 Potentially toxic trace elements (PTTEs) and nutrients

Analyzing potentially toxic trace elements (PTTEs) in algal-based products is crucial since seaweeds have a high capacity to absorb environmental contaminants (Agarwal et al., 2022). With the growing use of seaweed biofertilizers in sustainable agriculture, a thorough examination of their trace element content becomes essential to ensure the quality of the marketed products, as well as environmental and human health safety (Greger et al., 2007). Since *Kappaphycus* sp. is a potential biosorbent of potentially toxic elements (Rahman and

Sathasivam, 2015), this significant finding of no detectable PTTE contamination in the biostimulant samples produced in Southern Brazil supports its use in agriculture, aligning to utilize natural resources without introducing harmful pollutants into crop systems.

Regarding nutrients, K was the most abundant in all samples. Similar to our findings, K has been the most abundant macronutrient reported in the literature, with amounts ranging from 1.6 to 2.1% (Rathore et al., 2009; Zodape et al., 2009). In Brazil, Gelli et al. (2020) detected ~2% K in *K. alvarezii* grown in the sourtheastern region of the country. The use of algal extracts as a potassium source has improved the yield and quality of crop plants such as sugarcane (Karthikeyan and Shanmugam, 2017) and pak choi (Gangaiah et al., 2017). The amounts of the other macronutrients also varied according to season and the sites of production investigated, but their average values are in accordance with the literature (Rathore et al., 2009; Zodape et al., 2009; Layek et al., 2015; Gelli et al., 2020). Nevertheless, it's worth mentioning that, overall, PAL samples showed higher nutrient contents in respect to RIB ones, especially for calcium, magnesium, sulphur, and boron (PAL-5 = mid-spring) and organic C, phosphorus, copper and zinc (PAL 4 = late winter). This indicates that the small-scale environmental conditions in Palhoça farm (PAL) should have favored greater accumulation of those nutrients. Furthermore, the samples from late summer and early autumn from both sites (PAL-1, PAL-8, RIB-1, and RIB-8) presented the lowest values for almost all nutrients.

4.3 PCAs and Correlation Analysis

The biochemical PCA results indicate that carbohydrates (TC, TSS, TS) and amino acids (TAA) concentrations are more correlated with low temperatures, i.e., winter-collected samples (except RIB-4), such as PAL-2 (17.02°C) and PAL-5 (19.67°C). Interestingly, RIB-4 was the sample with the lowest carbohydrate content (Fig. 3), resembling the other summer-harvested ones. Also, nutrient PCA results demonstrate that most nutrients were more correlated with winter-collected samples and low-temperatures (PAL-2 and RIB-2), as well as carbohydrates (TC, TSS, and TS). The observed significant negative correlations between sea-surface temperature (SST) and key biochemical components suggest that these compounds accumulate more under lower temperatures. Additionally, the negative correlations observed with K, P, S, and Zn imply that nutrient uptake or retention is enhanced during colder periods.

4.4 Seasonal influences

Our results underscore the impact of seasonal variation on the biochemical and nutrient composition of *K. alvarezii*-based biostimulant, revealing distinct clusters on PCA plots corresponding to winter and summer periods. This indicates that environmental factors play a crucial role in influencing the biochemical and nutrient profiles of *K. alvarezii* grown in southern Brazil, with special emphasis on the effect of low temperatures. These variations are likely influenced by environmental factors over the seasons, particularly temperature, which directly affect the physiological processes of the seaweed and the synthesis of bioactive compounds (Kumar et al., 2021), specifically carbohydrate content, which has been negatively correlated with temperature, as also observed by Araújo et al (2022).

These findings highlight the importance of seasonality, particularly its influence on sea temperature, for the physiological state of the seaweeds (Kumar et al., 2020b). The observed changes in biochemical composition during the summer months may be attributed, at least in part, to the physiological responses of the seaweeds to elevated temperatures. As indicated by previous studies, temperatures exceeding 32-36°C can induce various stress responses in *K. alvarezii*, including decreased pigment content, reduced photosynthetic efficiency, diminished growth rates, and lower yields of carrageenan (Kumar et al., 2020b), and reduced nutrient uptake (Mandal et al., 2015).

In contrast, during colder periods such as late autumn, winter, and early spring in southern Brazil, the biostimulant samples presented greater abundance and homogeneity of biochemical compounds and nutrients, as evidenced by sample clusters detected by PCA. Nonetheless, it's important to note that low temperatures, particularly around 14°C, can reduce the photosynthetic capacity and damage the antioxidant and photosynthetic systems of *K. alvarezii*, thereby compromising its resilience and adaptation to stressful conditions (Li et al., 2016). In fact, seawater temperatures ≤ 14°C have been shown to severely impact the growth and survival of the seaweeds in the marine farms where they were cultivated (Silva, A. A. personal communication).

During winter, the coastal region of Santa Catarina State is significantly influenced by nutrient-rich currents such as the Malvinas Current (MC) and the Brazil Coastal Current (BCC), whereas throughout the rest of the year, it is primarily influenced by the Brazil Current (BC). BC is a southward-flowing current, relatively warm and saline, while MC is a northward-flowing current, characterized by cold, nutrient-rich, and low-salinity waters, due to its origin from Antarctic Circumpolar Current (Wainer et al., 2000). While BCC has high levels of

organic matter, discharged from the Patos Lagoon and La Plata River, that, on its way north, mixes with the other currents (Souza and Robinson, 2004). However, the maximal northward extension positions of the MC and BCC vary significantly between seasons (Piola et al., 2005; Piola and Matano, 2019), potentially influencing the coastal conditions during autumn and/or spring as well. The combination of low temperatures and nutrient influx from these currents during these seasons, likely enhances the accumulation of biochemical compounds and nutrients in *K. alvarezii*, and consequently, its biostimulant properties.

The results described herein highlight the *K. alvarezii*'s adaptation to seasonal changes, emphasizing the importance of considering temperature fluctuations and ocean currents in marine farms management. Furthermore, it is known that the microbiome associated with seaweeds plays a significant role in their cold resistance (Karsten et al., 1992). Therefore, understanding the endophytic microbiota of cultivated *K. alvarezii* could be a pivotal strategy. For instance, employing microbiome engineering may alleviate cold-induced growth issues (Ghaderiardakani et al., 2020) and could maintain the production of seaweeds and accumulation of bioactive compounds in the biostimulant, particularly during winter.

5. Conclusion

Our findings reveal notable variability in the biochemical and nutrient composition of the *K. alvarezii* biostimulant over the seasons in southern Brazil. Temperature fluctuations emerge as a key factor influencing this composition, with lower temperatures in winter and autumn promoting higher accumulation of bioactive compounds and some mineral nutrients. These results underscore the importance of considering seasonal oceanographic factors (e.g., seawater temperature, salinity, and nutrients) in optimizing cultivation practices. Moreover, to achieve product standardization and address future challenges, it is necessary to develop protocols that ensure consistent quality and efficacy despite fluctuating environmental conditions.

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Capítulo 2 – Caracterização físico-química e bioquímica do resíduo sólido industrial de *Kappaphycus alvarezii*

Chemical Profile of *Kappaphycus alvarezii* Residual Biomass from Biofertilizer Production: Seasonal Variation and Biorefinery Applications

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Abstract

Kappaphycus alvarezii is the world's primary source of κ-carrageenan and a versatile feedstock for food, nutraceutical, and agricultural applications. In Brazil, commercial production of its aqueous biostimulant extract generates a substantial solid residue whose seasonal and site-specific biochemical composition remains undocumented, limiting its valorization within circular bioeconomy frameworks. This study characterized the proximate composition, carbohydrate fractions, residual carrageenan yield, bioactive compounds (total phenolics, flavonoids, and carotenoids), macro- and micronutrients, and fatty-acid profile of the industrial solid residue obtained after ambient-temperature aqueous extraction of fresh *K. alvarezii* biomass cultivated across four seasons at two marine farms (Palhoça—PAL and Florianópolis—RIB) in Santa Catarina, southern Brazil. The residue retained high moisture (81.55–85.82%), elevated ash (25.37–31.87% dw, higher at PAL), stable lipids (mean 4.79%), substantial neutral detergent fiber (≤33.18%), total carbohydrates (≤537 mg g^{-1}), and residual κ-carrageenan (mean 17.87%, highest in Winter). Bioactive compounds persisted at nutritionally relevant levels, with site- and season-dependent patterns: PAL warm-season peaks in phenolics and flavonoids; RIB-winter in carotenoids. Principal component analysis resolved a seasonal axis (PC1, 27.8% variance) linked to carbohydrate and bioactive accumulation and a spatial axis (PC2, 19.1%) separating mineral-rich PAL from carotenoid-rich RIB samples, while Pearson correlations confirmed sea surface temperature as a key negative driver of ashes, soluble sugars, protein, and moisture. Fatty-acid profiles showed clear site-specific signatures (C24:0 exclusive to PAL; higher C17:1 at RIB). These results demonstrate that mild aqueous processing selectively preserves structural polysaccharides, minerals, and bioactive compounds, yielding a matrix suitable as a clean-label functional ingredient for fortification, texture modification, and antioxidant applications in foods and feeds. Seasonally optimized cascading biorefinery strategies are proposed to convert this otherwise discarded residue into value-added co-products. By linking environmental drivers to post-extraction composition, this work provides the scientific basis for year-round, site-tailored valorization, advancing zero-waste macroalgal processing and supporting sustainable blue-economy development in Brazil.

Keywords: bioactive compounds; carrageenan; circular economy; biochemical profile; red seaweed.

1 Introduction

The red macroalga *Kappaphycus alvarezii* (Doty) Doty is the world's primary industrial source of kappa-carrageenan, accounting for approximately 40% of global cultivated macroalgal tonnage and generating a market valued at over USD 1 billion annually [1,2]. Beyond carrageenan, the biochemical versatility of *K. alvarezii* — encompassing sulphated polysaccharides, phenolic compounds, carotenoids, and essential macro- and micronutrients — has underpinned over 200 documented applications across the food, pharmaceutical, agricultural, and cosmetic sectors [3,4]. In Brazil, the cultivation of *K. alvarezii* is expanding along the southeastern and southern coastline, where marine farmers produce an aqueous biostimulant extract from fresh biomass [5–7].

The post-extraction remaining biomass of *K. alvarezii* is far from inert; it retains a complex matrix of insoluble structural carrageenan, non-polar lipids, retained bioactive compounds (phenolics, carotenoids, flavonoids), and essential macro- and micronutrients [7]. A growing body of literature confirms that such processing co-products from macroalgae are not wasted materials but valuable feedstocks. For *K. alvarezii*, research has primarily focused on the remaining biomass from carrageenan extraction, demonstrating their potential for enzymatic glucose production for bioethanol, as well as for the extraction of cellulose for biopolymeric films [8–11]. More recently, the first characterization of the residual biomass specifically from aqueous extract production, has established its baseline biochemical value [7]. However, beyond this foundational study, the valorization of this specific biomass type remains severely underexplored. Critically, no studies have addressed the seasonal and site-specific dynamics that govern its biochemical composition, a gap that fundamentally hinders the development of predictable and economically viable year-round industrial applications.

Seasonal variation is a key determinant of biochemical composition in *K. alvarezii*, with environmental cues modulating the synthesis of carbohydrates, lipids, and secondary metabolites [12–14]. In southern Brazil, the pronounced seasonal gradient in sea surface temperature drives systematic fluctuations in the fresh biomass, with soluble sugars, lipids, and phenolics all exhibiting distinct seasonal peaks [14]. These dynamics are expected to propagate into the remaining biomass, influencing the availability of high-value fractions such as carrageenan, bioactive compounds, and nutrients. Without understanding this temporal variability, optimal harvest scheduling and the selection of appropriate downstream applications, whether for high-value bioactives in winter or carbohydrate-rich streams in summer, cannot be confidently defined. This knowledge gap is particularly critical for

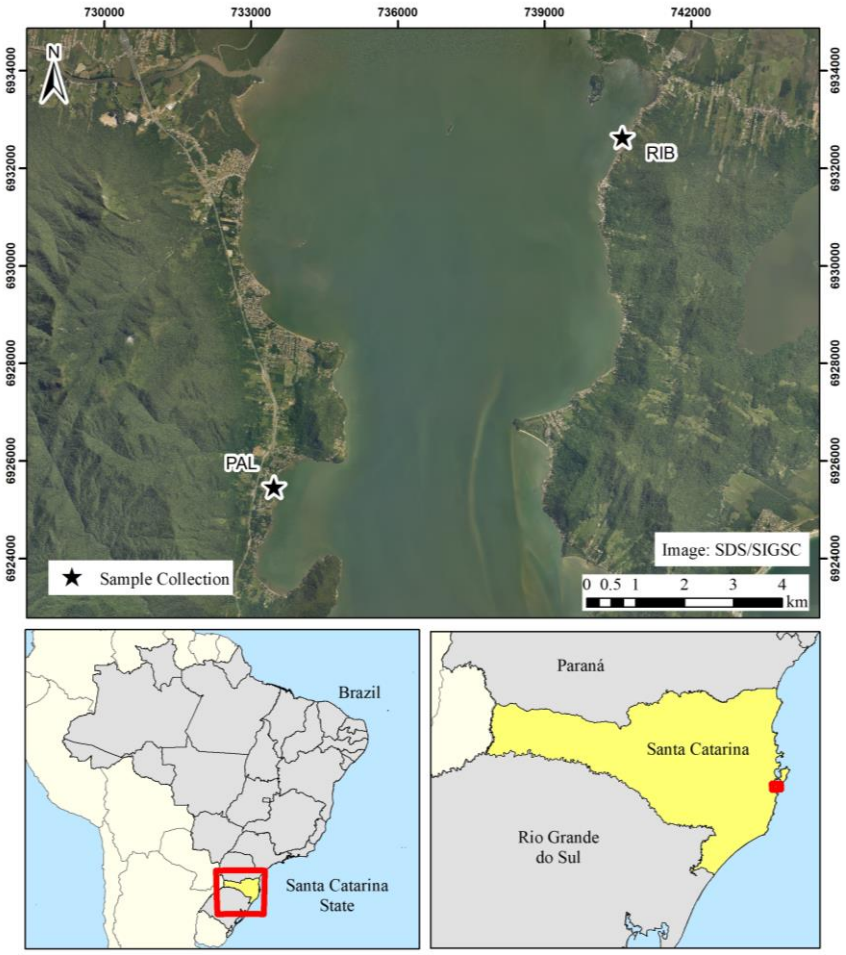
integrating such a co-product stream into a circular bioeconomy framework, which demands consistent and predictable feedstock characteristics.

In this context, the present study provides a comprehensive seasonal and site-specific biochemical characterization of the remaining biomass generated from industrial aqueous extract production of *K. alvarezii*, using algae biomass cultivated across four seasons at two marine farms in southern Brazil. By systematically analyzing proximate composition, carbohydrate fractions, retained carrageenan, bioactive compounds, and mineral and fatty acids profiles, this work aims to: (1) identify seasonal biochemical patterns in the remaining biomass; (2) evaluate its valorization potential within an integrated biorefinery framework, highlighting applications in functional foods, animal nutrition, and bio-based ingredients; and (3) propose seasonally-informed strategies for converting this co-product fraction into a value-added co-materials. This research directly advances the principles of a blue economy and circular bioeconomy by establishing the scientific basis for zero-waste algal processing, aligning with the United Nations Sustainable Development Goals for responsible consumption and production (SDG 12) and life below water (SDG 14) [15,16].

2 Materials e Methods

2.1 Sample collection

Samples of *K. alvarezii* remaining biomass (after algal pressing for aqueous extraction) were provided by two marine farms located in Santa Catarina state, southern Brazil: Ribeirão da Ilha beach, Florianópolis (RIB; 27° 42' 32.72" S, 48° 33' 35.50" W) and Palhoça (PAL; 27° 46' 29.93" S, 48° 37' 50.70" W) (Fig. 1). At each site, seaweeds were cultivated for three-month period prior to harvest and subsequent aqueous extraction. The remaining biomass fractions were systematically collected across all four seasons over a one-year cycle, mirroring the standard commercial production schedule [5].



Source: Nunes et al. [19].

Figure 1. Sample collection sites of the seaweed *Kappaphycus alvarezii* cultivated in sea farms in Florianópolis (RIB - Ribeirão da Ilha beach) and Palhoça (PAL) counties, state of Santa Catarina, southern Brazil.

The southern region of Brazil is in a subtropical zone and is classified as humid subtropical (Cfa) according to the Köppen climate classification. This region experiences hot summers, no distinct dry season, and average maximum temperatures above 22°C, with minimum averages ranging from -3°C to 18°C [17]. Precipitation is evenly distributed throughout the year, with a total annual rainfall of 2,016 mm recorded in 2023 in the Santa Catarina Island [18]. Because the seasonal variation of the environmental factors influences the physiology and biochemical composition of *K. alvarezii*, sampling was conducted across four seasons from April 2022 to March 2023. At each site, two samples were collected per season (mid- and late-season), resulting in a total of 32 samples (Table 1).

Part of the samples (500 g) were immediately placed in a drying oven (40 °C) with air circulation to be dried and stored for carrageenan extraction. Another part (~100 g) of each was

immediately placed in ziplock plastic bags and frozen in an ultrafreezer (-80°C) to be freeze-dried later. The choice for freeze-drying samples was made taking into account previous findings regarding the stabilization and optimization of the compound's recovery when extracted from the algal biomass [19].

Table 2 – Season, local, sample, ID, and date of *Kappaphycus alvarezii* sampling, and month average sea surface temperature (SST - °C) until date sampling. Source: Schneider et al. [14].

Season	Local	ID	Date	SST (°C)
Autumn	PAL	PAL-1	04/26/2022	22.44 (± 0.39)
		PAL-2	06/14/2022	17.02 (± 0.41)
	RIB	RIB-1	04/19/2022	26.05 (± 0.41)
		RIB-2	06/07/2022	22.96 (± 0.40)
Winter	PAL	PAL-3	07/26/2022	17.60 (± 0.39)
		PAL-4	09/20/2022	17.97 (± 0.40)
	RIB	RIB-3	07/19/2022	17.63 (± 0.38)
		RIB-4	09/13/2022	17.93 (± 0.39)
Spring	PAL	PAL-5	11/08/2022	19.67 (± 0.38)
		PAL-6	12/13/2022	22.05 (± 0.40)
	RIB	RIB-5	10/25/2022	19.07 (± 0.40)
		RIB-6	12/06/2022	22.78 (± 0.40)
Summer	PAL	PAL-7	02/07/2023	25.45 (± 0.40)
		PAL-8	03/14/2023	26.05 (± 0.41)
	RIB	RIB-7	01/24/2023	26.16 (± 0.40)
		RIB-8	03/07/2023	25.41 (± 0.40)

2.2 Moisture

For the determination of moisture in residue samples, initially, 20 g samples of each biomass were freeze-dried for approximately 20 h, at -50°C and 0.030 mbar (Labconco FreeZone 6 Liter Freeze Dryer). The moisture value was calculated by the following formula [20]:

$$M\% = \left[1 - \left(\frac{Wf}{Wi}\right)\right] x100$$

Where:

M% = Percentual moisture

Wi (Initial weight) = 20g

Wf (Final weight) = weight after freeze-drying.

2.3. Neutral Detergent Fiber (NDF)

The neutral detergent fiber (NDF) content of the dried residue samples was determined gravimetrically following the method described by Van Soest et al. [21], with adaptations. 400 mg of oven-dried sample was weighed, in triplicate, and enclosed in pre-weighed non-woven polypropylene bags, which were subsequently heat-sealed using an impulse sealer. The sealed bags were then immersed in a neutral detergent solution composed of sodium lauryl sulphate, disodium EDTA, sodium borate, dibasic sodium phosphate, and triethylene glycol (pH 7.0), and subjected to autoclave treatment at 121°C for 40 min to ensure complete solubilization of cell contents. Following the reaction, the bags were removed and rinsed thoroughly with abundant tap water, fallowed by three times with hot distilled water (70–80°C) to remove all soluble residues. The bags were oven-dried at 105°C for 12 h, allowed to cool in a desiccator, and reweighed. The NDF content was calculated as the difference between the final and initial bag masses, expressed as a percentage of the dry weight of the sample, representing the insoluble cell wall fraction comprising cellulose, hemicellulose, and lignin.

2.3 Ashes

Total ash content was determined gravimetrically following the AOAC Official Method, with adaptations for macroalgal biomass as recommended by Thiex et al. [22] and Liu [23]. Approximately 20 g of oven-dried residue sample was weighed into pre-weighed porcelain crucibles in triplicate. Prior to sample placement, the crucibles were pre-ignited at 600°C for 1 h, cooled in a desiccator, and weighed to constant mass to ensure the absence of hygroscopic residues. Dry samples were carried out at 600°C for 6 h until complete combustion of the organic fraction was achieved, as evidenced by the absence of black carbonaceous residue and the formation of a uniform white or grey ash. After incineration, crucibles were maintained in the furnace overnight to cool, and subsequently transferred to a desiccator until reaching room temperature. Crucibles were reweighed, and the total ash content was expressed as a percentage of the dry weight of the original sample, calculated as the mass difference between the crucible plus ash and the empty crucible, relative to the initial sample mass.

2.3 Biochemical analysis

The analyses of proximate composition (total protein, total lipids) and carbohydrates (total carbohydrates, total soluble sugars, total starch) were conducted for oven-dried samples, while bioactive components (phenolic content, flavonoid content, and total carotenoids) were analyzed in freeze-dried samples. All biochemical analyses, including fatty acid composition, were performed according to Schneider et al. [14], with the exception of total protein content. Absorbance measurements were carried out using a microplate reader (SpectraMax 190, Molecular Devices) with Softmax Pro 7 software. Blanks were prepared in the same manner as the samples, with the extraction agent replacing the sample.

2.3.1 Total Protein (TP)

The total protein content was determined using the Bradford [24] method with some adaptations. Briefly, 200 mg of oven-dried sample was extracted with 10 mL of phosphate-buffered saline (PBS) pH 7, vortexed, allowed to stand for 15 minutes, and centrifuged (10 min, 4000 rpm). An aliquot of 100 µL of the supernatant was mixed with 5 mL of a 1:5 dilution of the Bradford reagent in water and allowed to stand for 5 minutes. The absorbance was measured at 595 nm using a microplate reader (ThermoPlate®, model P-reader). A calibration curve was prepared using bovine serum albumin (Sigma-Aldrich, MO, USA) at concentrations ranging from 3.90 to 62.5 µg/mL ($y = 0.0032x$, $r2 = 0.9772$).

2.4 Carrageenan Yield

The retained carrageenan content was determined gravimetrically following a modified protocol based on Masarin et al. [8] and Webber et al. [25], with adaptations. Briefly, 1 g of oven-dried residue was immersed in 500 mL of 6% (w/v) KOH solution and heated at 60°C for 4 h to promote alkaline pre-treatment of the biomass. Following this step, the biomass was filtered and subjected to dialysis against distilled water for 24 h to ensure complete removal of residual KOH. The pre-treated biomass was then transferred to 500 mL of distilled water and heated at 80°C for 4 h under occasional stirring to promote polysaccharide solubilization. After 2 h, the algal material was manually macerated and reintroduced into the solution for a further 2 h. The resulting hydrogel was filtered under vacuum to remove insoluble impurities. Five aliquots of 50 mL from each sample were each mixed with 96% (v/v) ethanol at a 3:1 (ethanol:extract) ratio and 0.2% (w/v) KCl to induce carrageenan precipitation, and maintained at 4°C for 24 h. The precipitated carrageenan was recovered by filtration and dried at 40°C to

constant mass. The carrageenan yield was expressed as a percentage of the dry weight of the recovered carrageenan relative to the initial dry weight of the residue sample.

2.9 Statistical analysis

All biochemical variables (total protein, total lipids, neutral detergent fiber, total carbohydrates, total soluble sugars, total starch, total carotenoids, total phenolic compounds, total flavonoid compounds, moisture, and ash content) were determined in triplicate for each of the 16 sampling (eight from Palhoça — PAL, and eight from Florianópolis — RIB), yielding a total of 48 observations per variable. Carrageenan yield was determined in five replicates per sample (n = 5). Macro- and micronutrient contents were determined from a single composite sample per site (n = 16). Sea surface temperature (SST) was recorded as a single mean value per site, calculated from satellite-derived data over the 30 days preceding each sampling event.

Statistical comparisons were performed using a one-way design in which each sampling site was assigned to one of eight treatment groups defined by the combination of season and location (PAL-Autumn, PAL-Winter, PAL-Spring, PAL-Summer, RIB-Autumn, RIB-Winter, RIB-Spring, and RIB-Summer). The normality of residuals was assessed using the Shapiro–Wilk test and homogeneity of variances was evaluated using Levene's test, both at $\alpha = 0.05$. Variables satisfying both assumptions were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post-hoc test. Variables violating at least one assumption were analyzed using the Kruskal–Wallis H test followed by Dunn's post-hoc test with Bonferroni correction for all 28 pairwise comparisons. Statistical significance was set at $p < 0.05$ for all tests. Results are presented as mean ± standard deviation. Compact letter displays (CLD) were used to summarize pairwise comparisons, with groups sharing a letter not differing significantly.

Pearson's correlation coefficients were computed pairwise among SST, all 11 biochemical variables, carrageenan yield and the 11 macro- and micronutrients. Correlations involving biochemical variables and carrageenan were computed using the full replicated dataset (n = 48), while those involving nutrients used the site-level mean values (n = 16). For mixed correlations between biochemical and nutrient variables, the single nutrient value per site was matched to all three biochemical replicates of that site; these correlations should therefore be interpreted with caution, as the effective sample size is 16 rather than 48.

Principal component analysis (PCA) was performed separately for the biochemical dataset (12 variables, site means, n = 16), the nutrient dataset (11 variables, n = 16), and the

fatty acid dataset (9 variables, n = 16; see below). In all cases, variables were standardized to zero mean and unit variance prior to analysis, and principal components were extracted using singular value decomposition (SVD). Score biplots (PC1 × PC2) were constructed and seasonal trends were visualized using 95% confidence ellipses drawn from eigenvalue decomposition of the within-group covariance matrix. Nutrient concentrations were additionally visualized as a z-score heatmap, with rows corresponding to nutrients and columns to sampling sites ordered by season, with cell color reflecting the standardized concentration per variable.

For the fatty acid (FA) dataset, mean relative area values, expressed as a percentage of the total chromatogram area, were calculated for each of the 16 samples, and fatty acids detected in fewer than four sites were excluded from the analysis, totalizing 9 FAs. A heatmap was constructed with sites ordered by season and locations interleaved within each season; values were standardized by z-score normalization calculated independently per row prior to visualization. PCA was subsequently performed on the same nine-variable dataset, with variables standardized to zero mean and unit variance, and principal components extracted by singular value decomposition (SVD). A total of 16 samples were considered.

All statistical analyses and data visualizations were performed in R (version 4.5.2) using the following packages: *readxl* [26] for data import; *tidyverse* [26] for data manipulation and reshaping; *car* [27] for Levene's test; *dunn.test* [28] for Dunn's post-hoc test; *multcompView* [29] for compact letter displays; *ggplot2* [30] for all graphical outputs; *ggrepel* [31] for non-overlapping sample labels in biplots; and *openxlsx* [32] for exporting statistical results.

3 Results

3.1 Proximate composition

Moisture content ranged from 81.55 ± 2.20% (RIB-Autumn) to 85.82 ± 2.29% (RIB-Spring). The only difference observed was within Spring, RIB (85.82 ± 2.29%) presented significantly higher moisture than PAL (82.20 ± 0.63%) ($H = 16.367$, $p = 0.022$). No significant seasonal and local variation was observed (Figure 2A). Ash content ranged from 25.37 ± 1.56% (RIB-Summer) to 31.87 ± 1.42% (RIB-Winter). A consistent and significant location effect throughout the year, with PAL samples maintained high and relatively stable ash contents across all seasons (30.35–31.87%), whereas RIB showed a progressive decline from Autumn (29.48 ± 0.77%) through to Summer (25.37 ± 1.56%) ($H = 37.812$, $p < 0.001$) (Figure 2C).

Total protein (TP) content ranged from 0.40 ± 0.16 mg g^{-1} (RIB-Summer) to 1.09 ± 0.07 mg g^{-1} (RIB-Winter). RIB samples varied significantly across the seasons: RIB-Winter

was higher than RIB-Summer ($H = 28.947$, $p < 0.001$). Within PAL, TP did not vary significantly across seasons (0.53–0.74 mg g^{-1}). During Winter, RIB, were higher than PAL, whereas during Summer, the opposite was observed (Figure 2C). Total lipid (TL) content ranged from 3.90% ± 1.12% (RIB-Autumn) to 5.43% ± 1.36% (PAL-Summer) but did not differ significantly among Season or site ($F = 0.862$, $p = 0.544$), with overall mean of 4.79% ± 1.20% (Figure 2D).

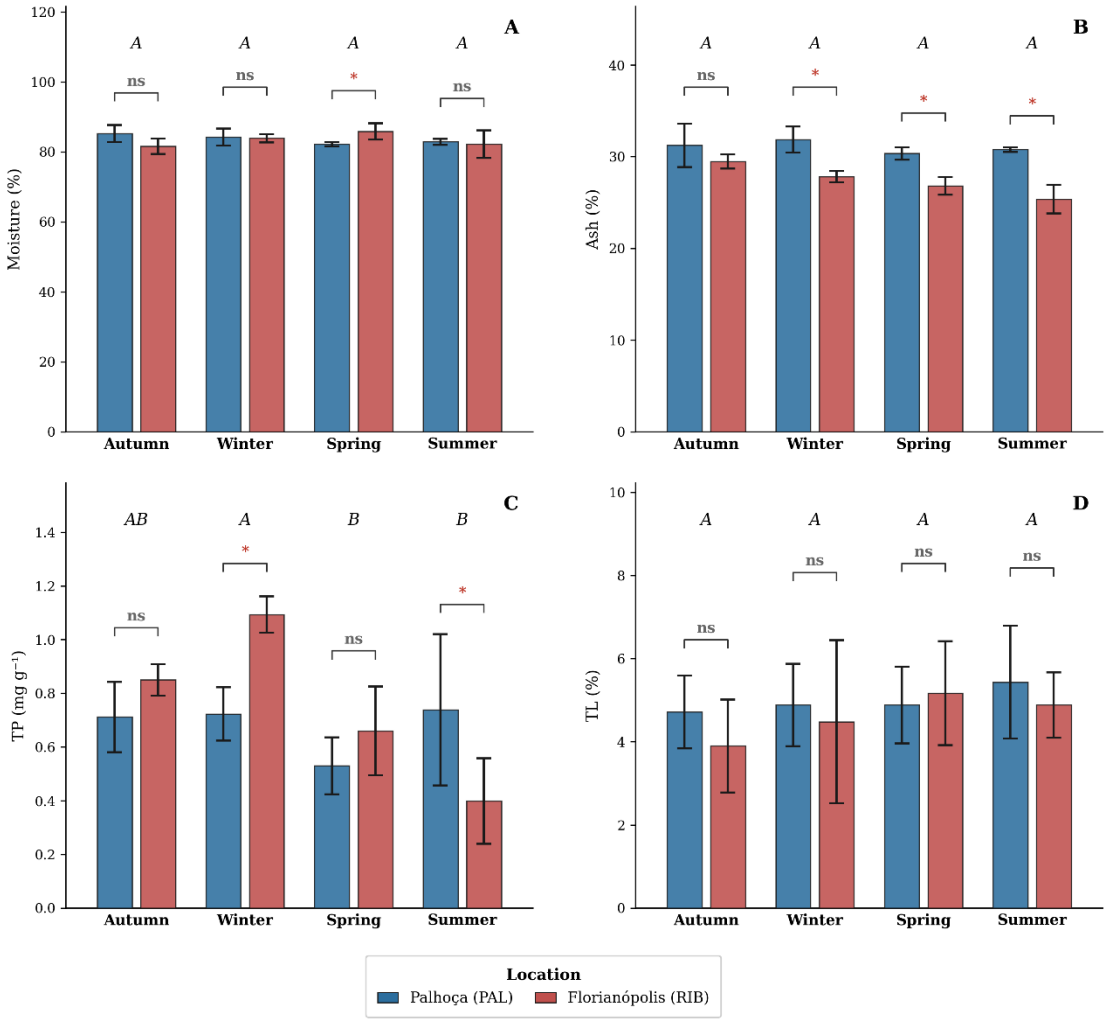


Figure 2 - Mean ± standard deviation of proximate composition of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production, cultivated in Palhoça (PAL) and Florianópolis (RIB), Santa Catarina, Brazil, sampled across four seasons over one year. (A) Moisture content; (B) Ash content; (C) Total protein (TP); (D) Total lipids (TL). Different uppercase letters above each seasonal group indicate significant differences among seasons (Tukey HSD or Dunn–Bonferroni post-hoc test, $p < 0.05$). Brackets with asterisks (*) indicate significant differences between locations within each season (Student's t-test, Welch's t-test, or Mann–Whitney U test, $p < 0.05$); ns = not significant.

3.2 Carbohydrate fractions

Neutral detergent fiber (NDF) ranged from 9.17 ± 2.15% (RIB-Winter) to 33.18 ± 8.70% (PAL-Spring) ($H = 22.325$, $p = 0.002$). No systematic seasonal trend was identified within either sites independently. Comparing between sites, PAL were higher than PAL in Winter and Spring, and no differences were observed on Autumn and Summer (Figure 3A). Total carbohydrates (TC) ranged from 238.00 ± 68.86 mg g^{-1} (RIB-Spring) to 537.00 ± 28.00 mg g^{-1} (RIB-Autumn). The most consistent seasonal pattern was observed within RIB, where Autumn presented significantly higher TC than both Spring and Summer ($H = 29.066$, $p < 0.001$). No significant differences were detected among PAL seasonal groups (Figure 3B).

Total soluble sugars (TSS) ranged from 1.16 ± 0.34 mg g^{-1} (RIB-Summer) to 8.15 ± 3.80 mg g^{-1} (PAL-Autumn). Within RIB, Summer presented significantly lower TSS than both Winter ($p = 0.019$) and Spring ($p = 0.048$) ($H = 18.220$, $p = 0.011$). No significant seasonal differences were detected within PAL, where TSS values remained moderate and variable throughout the year. No differences were observed between sites within each season (Figure 3C). Total starch (TS) ranged from 78.70 ± 13.00 mg g^{-1} (PAL-Spring) to 266.17 ± 132.20 mg g^{-1} (RIB-Summer) and differed markedly among groups ($H = 34.46$, $p < 0.001$). Spring was the globally depleted group (overall mean = 79.98 ± 12.63 mg g^{-1}), differing significantly from the other seasons (Autumn = 220.78 ± 47.04 mg g^{-1}, Winter = 224.08 ± 68.43 mg g^{-1}, Summer = 211.94 ± 107.74 mg g^{-1}). No significant differences were detected among PAL seasons or among non-Spring RIB groups. Comparing the sites, RIB samples presented higher values of starch than PAL during Autumn and Winter (Figure 3D).

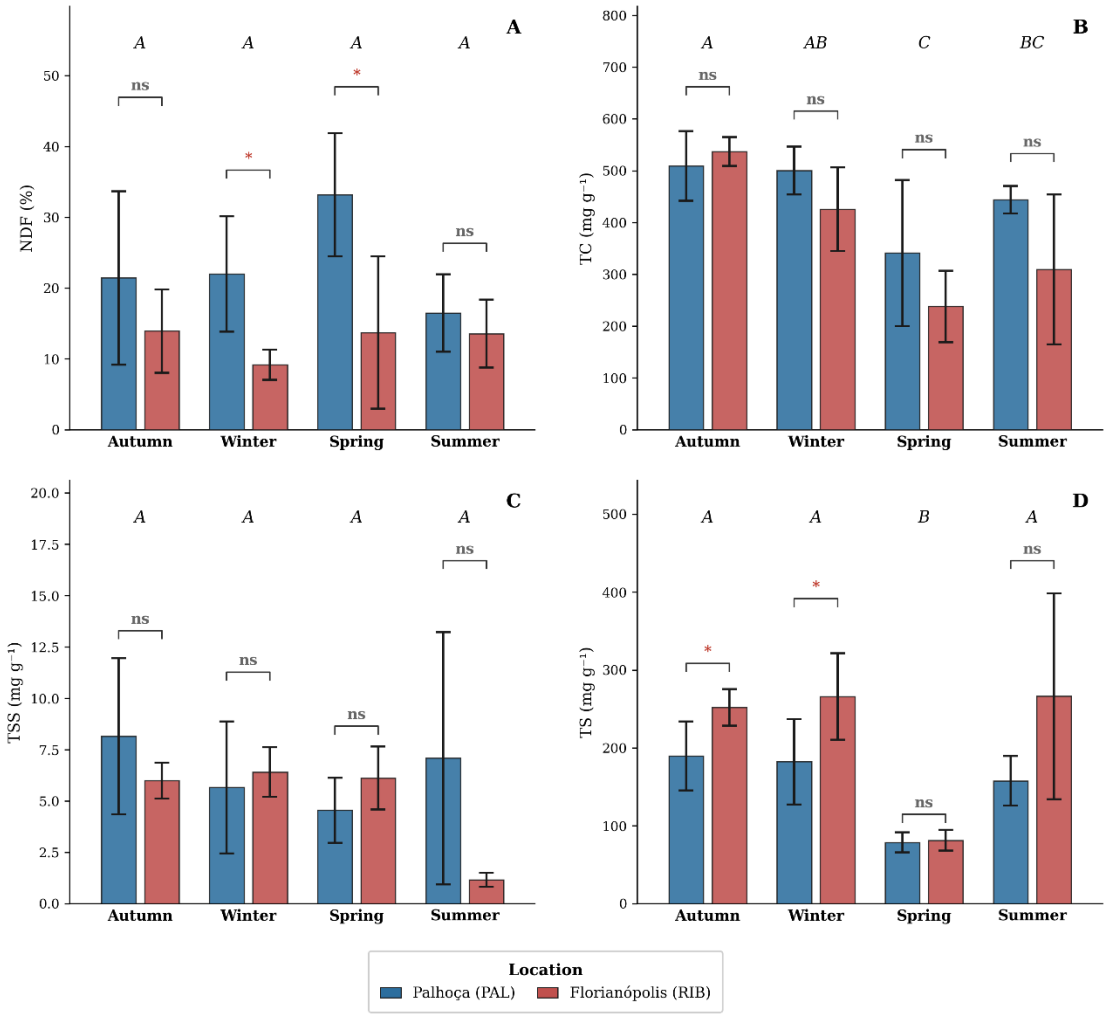


Figure 3 - Mean ± standard deviation of carbohydrate fraction of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production, cultivated in Palhoça (PAL) and Florianópolis (RIB), Santa Catarina, Brazil, sampled across four seasons over one year. (A) Neutral detergent fiber (NDF); (B) Total carbohydrates (TC); (C) Total soluble sugars (TSS); (D) Total starch (TS). Different uppercase letters above each seasonal group indicate significant differences among seasons (Tukey HSD or Dunn–Bonferroni post-hoc test, $p < 0.05$). Brackets with asterisks (*) indicate significant differences between locations within each season (Student's t-test, Welch's t-test, or Mann–Whitney U test, $p < 0.05$); ns = not significant.

3.3 Carrageenan yield

Carrageenan yield ranged from 12.25 ± 2.27% (PAL-Autumn) to 22.98 ± 6.38% (RIB-Winter), with an overall mean of 17.87 ± 6.63%. PAL-Autumn was significantly inferior to PAL-Winter and RIB-Winter ($F = 3.448$, $p = 0.003$). No significant between-location differences were detected in any season other than Autumn, where PAL (12.25 ± 2.27%) was notably lower than RIB (17.46 ± 3.91%) (Figure 4A).

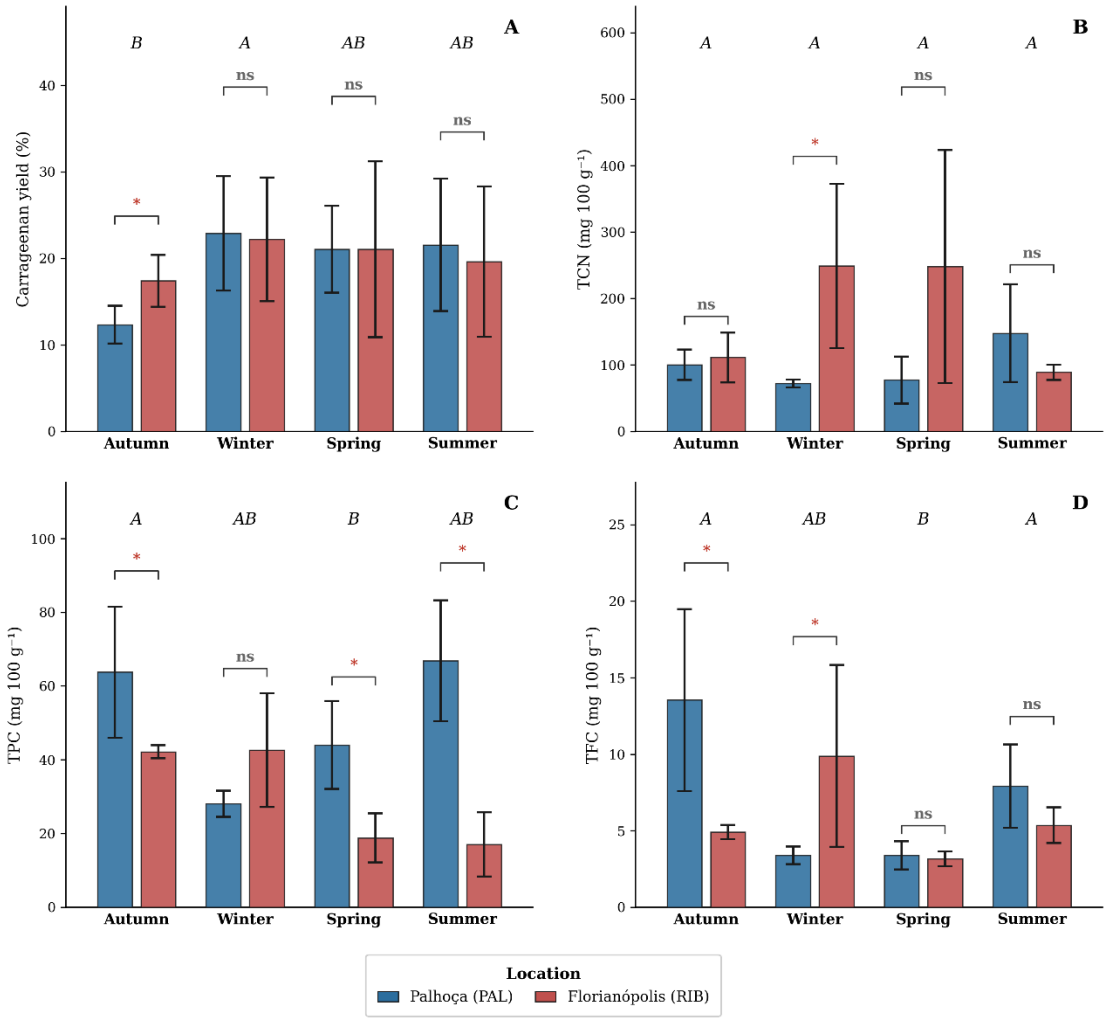


Figure 4 - Mean ± standard deviation of carrageenan yield and bioactive compounds of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production, cultivated in Palhoça (PAL) and Florianópolis (RIB), Santa Catarina, Brazil, sampled across four seasons over one year. (A) Carrageenan yield; (B) Total carotenoids (TCN); (C) Total phenolic compounds (TPC); (D) Total flavonoid compounds (TFC). Different uppercase letters above each seasonal group indicate significant differences among seasons (Tukey HSD or Dunn–Bonferroni post-hoc test, $p < 0.05$), with A denoting the highest mean. Brackets with asterisks (*) indicate significant differences between locations within each season (Student's t-test, Welch's t-test, or Mann–Whitney U test, $p < 0.05$); ns = not significant.

3.4 Bioactive compounds

Total carotenoid content (TCN) ranged from 72.08 ± 6.02 mg 100g^{-1} (PAL-Winter) to 248.96 ± 123.52 mg 100g^{-1} (RIB-Winter) and differed significantly among groups ($H = 23.174$, $p = 0.002$). Overall, no differences were observed between Seasons. Within Winter, RIB (248.96 ± 123.52 mg 100g^{-1}) recorded more than three times the carotenoid content of PAL

(72.08 ± 6.02 mg 100g^{-1}). No differences were observed between sites on the other seasons (Figure 4B).

Total phenolic compounds (TPC) ranged from 17.05 ± 8.71 mg 100 g^{-1} (RIB-Summer) to 66.83 ± 16.35 mg 100 g^{-1} (PAL-Summer). The values differed significantly within Season and Site ($H = 35.45$, $p < 0.001$). PAL-Autumn (63.75 ± 17.75 mg 100g^{-1}) was significantly higher than both RIB-Spring (18.78 ± 6.66 mg 100g^{-1}; $p = 0.003$) and RIB-Summer (17.05 ± 8.71 mg 100g^{-1}; $p = 0.003$); and PAL-Summer (66.83 ± 16.35 mg 100g^{-1}) was similarly higher than RIB-Spring ($p = 0.002$) and RIB-Summer ($p = 0.002$). No significant differences were found among PAL seasonal groups, nor among RIB seasonal groups (Figure 4C).

Total flavonoid content (TFC) ranged from 3.16 ± 0.49 mg 100 g^{-1} (RIB-Spring) to 13.54 ± 5.94 mg 100 g^{-1} (PAL-Autumn), with significant difference ($H = 33.73$, $p < 0.001$); PAL-Autumn was significantly higher than PAL-Winter (3.39 ± 0.59 mg 100g^{-1}), PAL-Spring (3.39 ± 0.93 mg 100g^{-1}). PAL-Summer (7.92 ± 2.72 mg 100g^{-1}). No significant differences were detected within RIB or in any other between-location comparison.

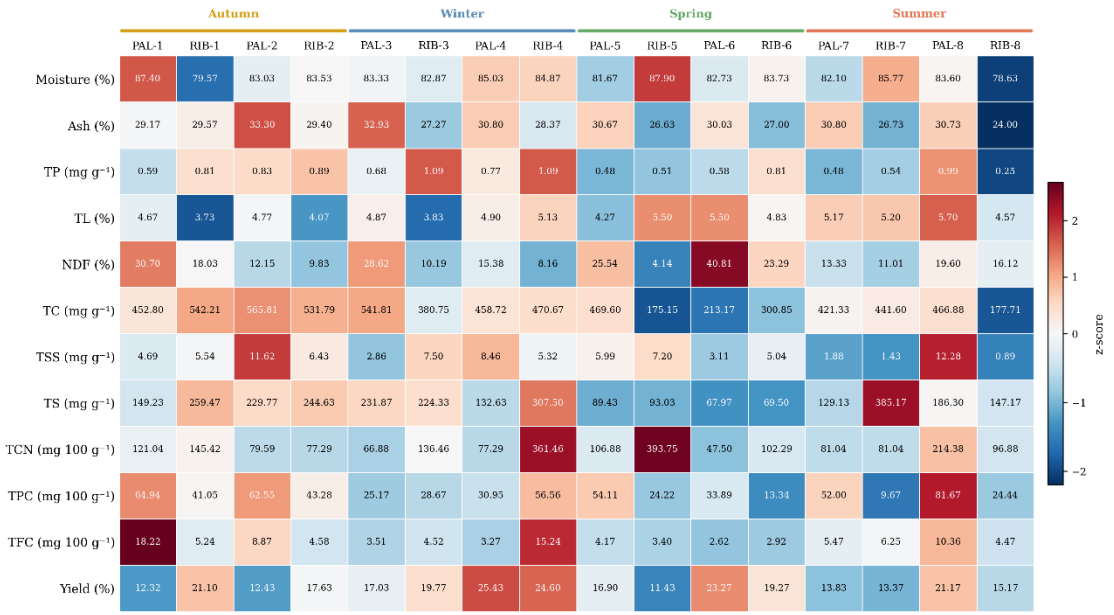


Figure 5 - Heatmap of biochemical composition of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production, cultivated in Palhoça (PAL) and Florianópolis (RIB), Santa Catarina, Brazil, sampled across four seasons over one year. Rows represent biochemical variables and columns represent sampling sites, ordered by season (Autumn, Winter, Spring, and Summer) with PAL and RIB interleaved within each season. Cell color indicates the relative concentration expressed as z-score per variable, ranging from blue (high) to red (low), enabling visual comparison across variables of different scales and units. Numerical values represent raw means of three analytical replicates per site. Variable

abbreviations and units: Moisture (%), Ash, Ashes (%); TP, total protein (mg g^{-1} dry matter); TL, total lipids (% dry matter); NDF, neutral detergent fiber (% dry matter); TC, total carbohydrates (mg g^{-1} dry matter); TSS, total soluble sugars (mg g^{-1} dry matter); TS, total starch (mg g^{-1} dry matter); TCN, total carotenoids (mg 100 g^{-1} dry matter); TPC, total phenolic compounds (mg 100 g^{-1} dry matter); TFC, total flavonoid compounds (mg 100 g^{-1} dry matter); Yield, carrageenan yield (%).

3.5 Principal Components Analysis (PCA) of Biochemical Compounds

Principal component analysis (Figure 6) of the 12 variables retained three components explaining a cumulative variance of 62.4% (PC1 = 27.8%, PC2 = 19.1%, PC3 = 15.4%). PC1 separated samples primarily along a gradient of carbon-rich compounds versus structural polysaccharides and carrageenan yield: TC, TPC, TP, TSS, TFC, TS, and Ash loaded positively, while NDF and carrageenan yield loaded negatively. PC2 was largely driven by location, with TCN, Moisture, and TL contributing positively, broadly associated with RIB samples, and Ash, NDF, and TC contributing negatively, associated with PAL samples; mean PC2 scores confirmed this spatial structuring (PAL: −0.70; RIB: +0.70). The seasonal trajectory was most evident along PC1, where Autumn samples presented the highest mean scores (+1.47) and Spring the lowest (−1.52), consistent with the strong carbohydrate depletion observed in Spring at both locations. Together, the biplot indicates that the biochemical composition of the residue is co-shaped by two partially independent axes: a seasonal axis (PC1) governing carbohydrate and bioactive accumulation, and a spatial axis (PC2) reflecting constitutive differences in mineral content (ashes), moisture, and carotenoid accumulation between the two cultivation sites.

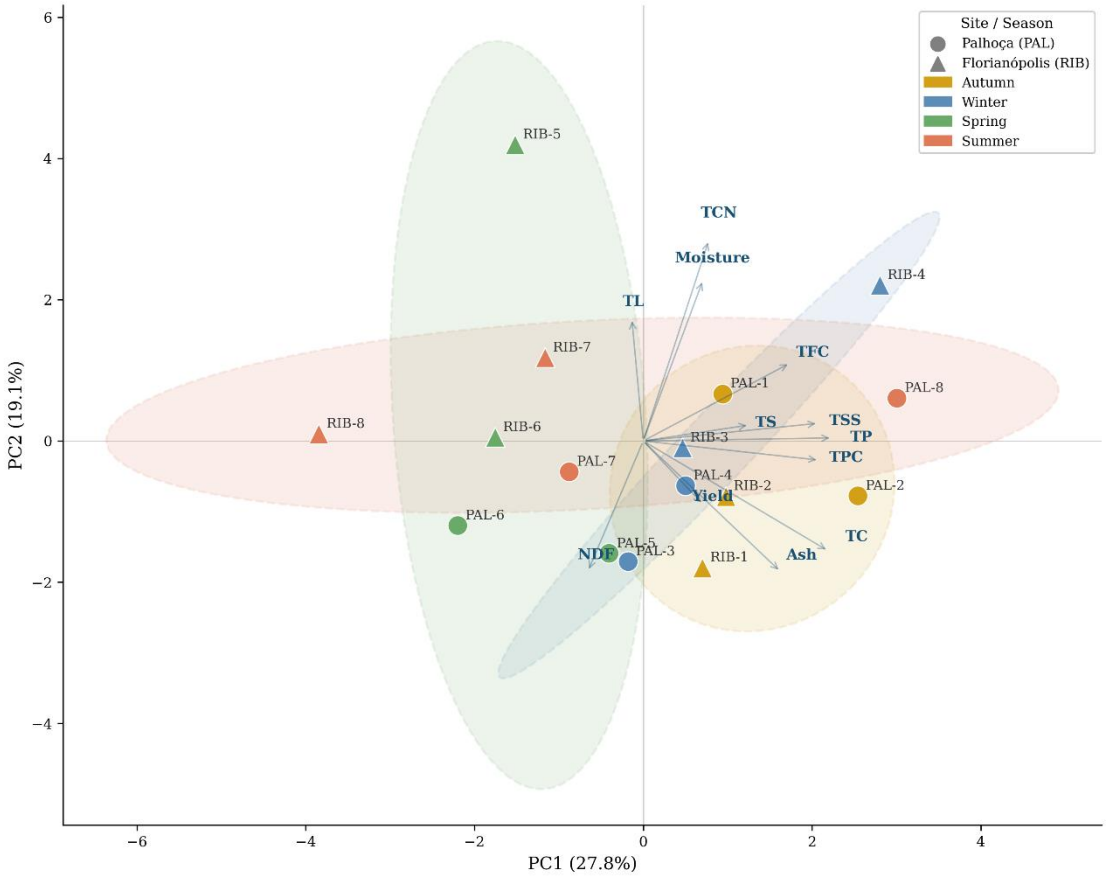


Figure 6 - Score plot of the first two principal components (PC1 × PC2) derived from principal component analysis (PCA) of the biochemical composition of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production, cultivated in Palhoça (PAL, circles) and Florianópolis (RIB, triangles), Santa Catarina, Brazil, sampled across four seasons over one year. Arrows represent variable loadings; arrow length and direction indicate the contribution and association of each variable with the principal components. Ellipses represent 95% confidence intervals for each location group. Abbreviations: Ash, ash content; TP, total protein; TL, total lipids; NDF, neutral detergent fiber; TC, total carbohydrates; TSS, total soluble sugars; TS, total starch; TCN, total carotenoids; TPC, total phenolic compounds; TFC, total flavonoid compounds; Yield, carrageenan yield.

3.6 Macro- and Micronutrients

The macronutrient profile of the residual biomass revealed distinct seasonal and spatial patterns across the 16 sampling, with sulphur (S) as the dominant element, followed by calcium (Ca), potassium (K), nitrogen (N), phosphorus (P), and magnesium (Mg) (Figure 7). Sulphur (S) ranged from 38.19 to 56.11 mg g^{-1} and displayed a clear seasonal trend, with the highest concentrations in Spring (47.48–56.11 mg g^{-1}) and winter (44.28–50.43 mg g^{-1}). Calcium (Ca) varied between 32.05 and 49.62 mg g^{-1} and was notably elevated from late winter to early summer in both locations. Peak values were recorded at PAL-7 (early summer; 49.62 mg g^{-1}),

PAL-5 and RIB-5 (early spring; 48.62 mg g^{-1}), RIB-6 (late spring; 48.62 mg g^{-1}), and PAL-4 (late winter; 45.62 mg g^{-1}). Potassium (K) concentrations (9.57–18.83 mg g^{-1}) differed markedly between locations. In PAL samples, a peak was observed at PAL-5 (early spring; 14.45 mg g^{-1}), while the remaining samples showed little variation (9.57–12.11 mg g^{-1}). In RIB samples, K was consistently higher from late winter to late Summer (17.19–17.36 mg g^{-1}) compared to Autumn and early winter (9.99–11.74 mg g^{-1}). With the exception of late Autumn, all RIB samples exhibited higher K values than their PAL counterparts. Nitrogen (N) ranged from 7.28 mg g^{-1} (PAL-5) to 15.82 mg g^{-1} (PAL-8). RIB consistently presented higher N concentrations, with the highest values in late autumn (14.98 mg g^{-1}) and throughout winter (13.72–14.98 mg g^{-1}).

Among the micronutrients, iron (Fe) exhibited the highest values, ranging from 82.31 mg kg^{-1} to 282.94 mg kg^{-1}, with the highest concentrations observed at RIB-Winter samples (RIB-3: 282.94 mg kg^{-1}; RIB-4: 263.14 mg kg^{-1}) and at PAL-7 (210.28 mg kg^{-1}). Boron (B), the second most abundant, with values ranged from 84.46 to 132.55 mg kg^{-1}, with Summer and late Spring recording the highest values (RIB-7: 132.55 mg kg^{-1}; RIB-6: 130.24 mg kg^{-1}; RIB-8: 124.15 mg kg^{-1}), while early Autumn samples were consistently the lowest (PAL-1: 85.57; RIB-1: 85.77 mg kg^{-1}). Manganese (Mn) followed a similar Fe pattern, with elevated values in Winter (RIB-3: 20.86 mg kg^{-1}; PAL-4: 19.70 mg kg^{-1}; RIB-4: 18.18 mg kg^{-1}) and Summer (PAL-7: 19.70 mg kg^{-1}; RIB-7: 17.39 mg kg^{-1}). Zinc (Zn) values range from 8.11 mg kg^{-1} (RIB-8) to 36.41 mg kg^{-1} (PAL-4), with no distinguishing seasonal or location pattern. Copper (Cu) showed a heterogeneous distribution with no clear seasonal pattern, ranging from 0.73 mg kg^{-1} (PAL-1) to 4.80 mg kg^{-1} (PAL-2).

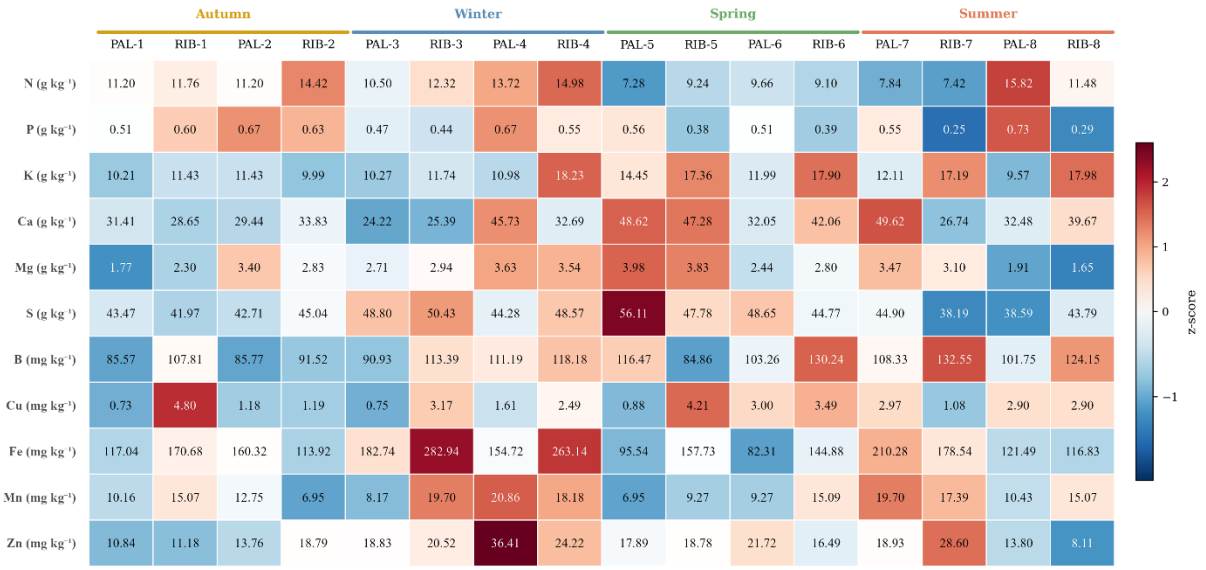


Figure 7 - Heatmap of macro- and micronutrient contents of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production, cultivated in Palhoça (PAL) and Florianópolis (RIB), Santa Catarina, Brazil, sampled across four seasons over one year. Rows represent nutrients and columns represent sampling sites, ordered by season (Autumn, Winter, Spring, Summer) with PAL and RIB interleaved within each season. Cell color indicates the relative concentration expressed as z-score per nutrient, ranging from blue (low) to red (high), enabling comparison across variables of different scales and units. Numerical values represent raw concentrations: macronutrients (N, P, K, Ca, Mg, S) are expressed as mg g^{-1} and micronutrients (B, Cu, Fe, Mn, Zn) as mg kg^{-1} dry matter. PAL, Palhoça; RIB, Florianópolis.

Principal component analysis of the 11 macro- and micronutrient variables yielded three components accounting for a cumulative variance of 64.4% (PC1 = 27.9%, PC2 = 18.7%, PC3 = 17.8%). The score plot revealed a partial seasonal structuring. Autumn samples from both locations (PAL-1, PAL-2, RIB-1, RIB-2) tended to cluster together in PC1- and PC2+PC2-, consistent with the low levels of all nutrient concentrations, but P. The Winter samples RIB-3, RIB-4, and PAL-4, and RIB-Summer samples RIB-7 and RIB-8, grouped in PC1+ and PC2+, along with Fe, Mn, Cu, B, and Zn. Summer and Spring samples showed great dispersion across the biplot, reflecting the divergent nutrient profiles between the two locations in those seasons.

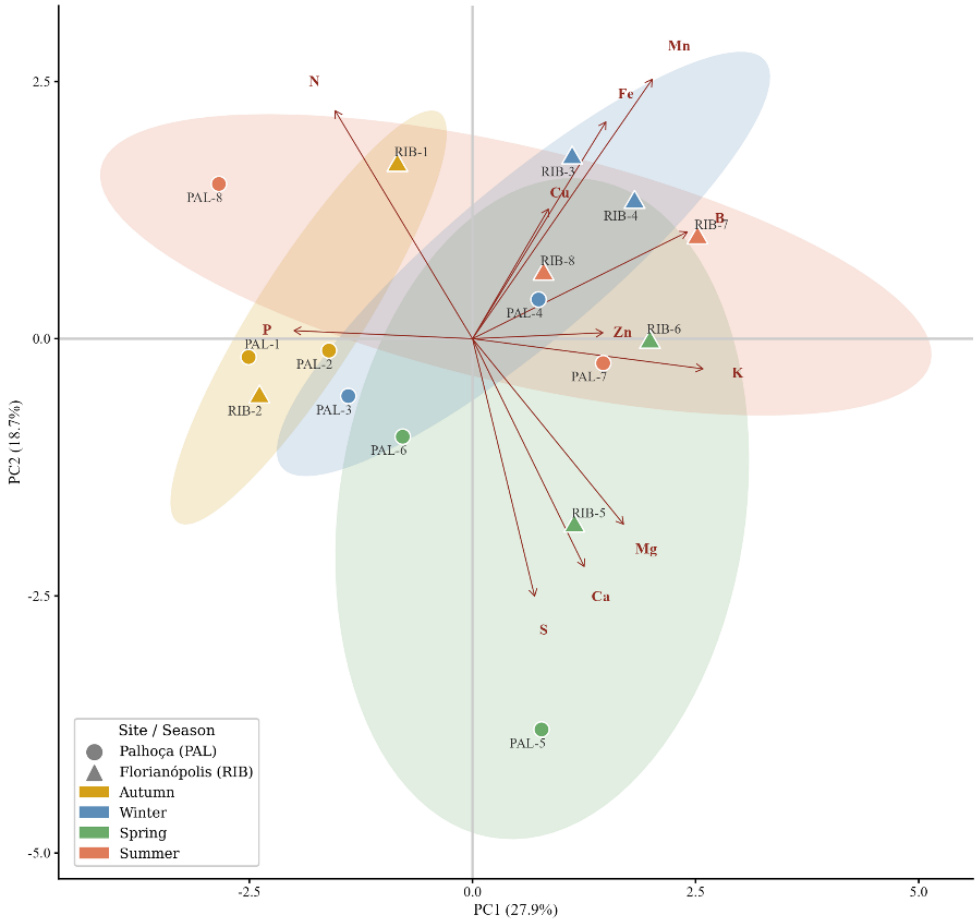


Figure 8 - Score plot of the first two principal components (PC1 × PC2) derived from principal component analysis (PCA) of the biochemical composition of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production, cultivated in Palhoça (PAL, circles) and Florianópolis (RIB, triangles), Santa Catarina, Brazil, sampled across four seasons over one year. Arrows represent variable loadings; arrow length and direction indicate the contribution and association of each variable with the principal components. Ellipses represent 95% confidence intervals for each location group. Abbreviations: N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; S, sulphur; B, boron; Cu, copper; Fe, iron; Mn, manganese; Zn, zinc.

3.7 Pearson Correlation Analysis

The Pearson correlation matrix revealed a broad network of significant associations among SST, biochemical variables, and macro- and micronutrients of the industrial solid residue of *K. alvarezii* (Figure 9). SST exerted a consistent negative influence on several biochemical variables, with significant inverse correlations observed for ash content ($r = -0.329$, $p = 0.022$), TSS ($r = -0.341$, $p = 0.018$), TP ($r = -0.320$, $p = 0.027$), and moisture ($r = -0.299$, $p = 0.039$), indicating that warmer water temperatures are associated with reductions

in mineral content, soluble sugars, protein, and water retention in the residue. Among macronutrients, SST showed a negative association with N ($r = -0.341$, $p < 0.05$) and P, consistent with the pattern of higher N concentrations observed in cooler seasons at RIB, and a positive association with K ($r = +0.312$, $p < 0.05$), reflecting the seasonal accumulation of potassium towards Spring and Summer described above. SST was not significantly correlated with TL, NDF, TC, TS, TCN, TPC, TFC, carrageenan yield, or the micronutrients B, Cu, Fe, Mn, and Zn, suggesting that these fractions are regulated by factors beyond sea surface temperature alone, such as photoperiod, nutrient availability, or site-specific hydrodynamics.

Among biochemical variables, the strongest correlation observed was between TC and ash content ($r = +0.661$, $p < 0.001$), suggesting that both variables are maximized under cooler, high-mineral-availability conditions and share a common physiological basis. TS and TC were also strongly and positively correlated ($r = +0.546$, $p < 0.001$), consistent with starch representing a major component of the total carbohydrate pool. TP showed positive associations with TSS ($r = +0.567$, $p < 0.001$) and TC ($r = +0.449$, $p < 0.001$), which may reflect a coupled anabolic state in which carbon and nitrogen resources are simultaneously mobilized during periods of active growth. TPC and TFC were strongly inter-correlated ($r = +0.574$, $p < 0.001$), as expected given that flavonoids are a subclass of phenolic compounds, and both showed positive associations with ash content, possibly reflecting the role of mineral elements as cofactors in phenylpropanoid biosynthesis. TCN was negatively correlated with NDF ($r = -0.446$, $p < 0.001$) and positively with moisture ($r = +0.386$, $p < 0.05$), suggesting that carotenoid accumulation is favored in samples with higher water content and reduced structural fiber density, conditions potentially associated with younger or more metabolically active thallus tissue.

Among the macro- and micronutrients, several ecologically and physiologically relevant associations were identified. Ca showed a strong positive correlation with Mg ($r = +0.512$, $p < 0.001$) and S ($r = +0.487$, $p < 0.001$), reflecting the co-accumulation of divalent cations and sulphur in Spring, a period characterized by active cell wall synthesis and sulphated polysaccharide production in red algae. Fe and Mn were positively correlated ($r = +0.623$, $p < 0.001$), consistent with their shared geochemical behavior in marine sediments and seawater, and both showed positive associations with TS, suggesting that periods of high starch accumulation coincide with elevated iron and manganese uptake. Zn was positively correlated with Fe ($r = +0.445$, $p < 0.01$) and Mn, reinforcing the pattern of co-accumulation of these transition metals in Winter samples. N showed a negative association with K ($r = -0.489$, $p <$

0.001), reflecting the opposing seasonal dynamics of these two macronutrients — N peaking in cooler months and K in warmer ones — and a positive correlation with TP ($r = +0.398$, $p < 0.05$), as expected from the biochemical relationship between nitrogen availability and protein synthesis. It should be noted that, as nutrient values represent single measurements per site replicated across three biochemical subsamples, correlations involving nutrients may be subject to a degree of inflation relative to those computed entirely from replicated data, and should be interpreted with corresponding caution.

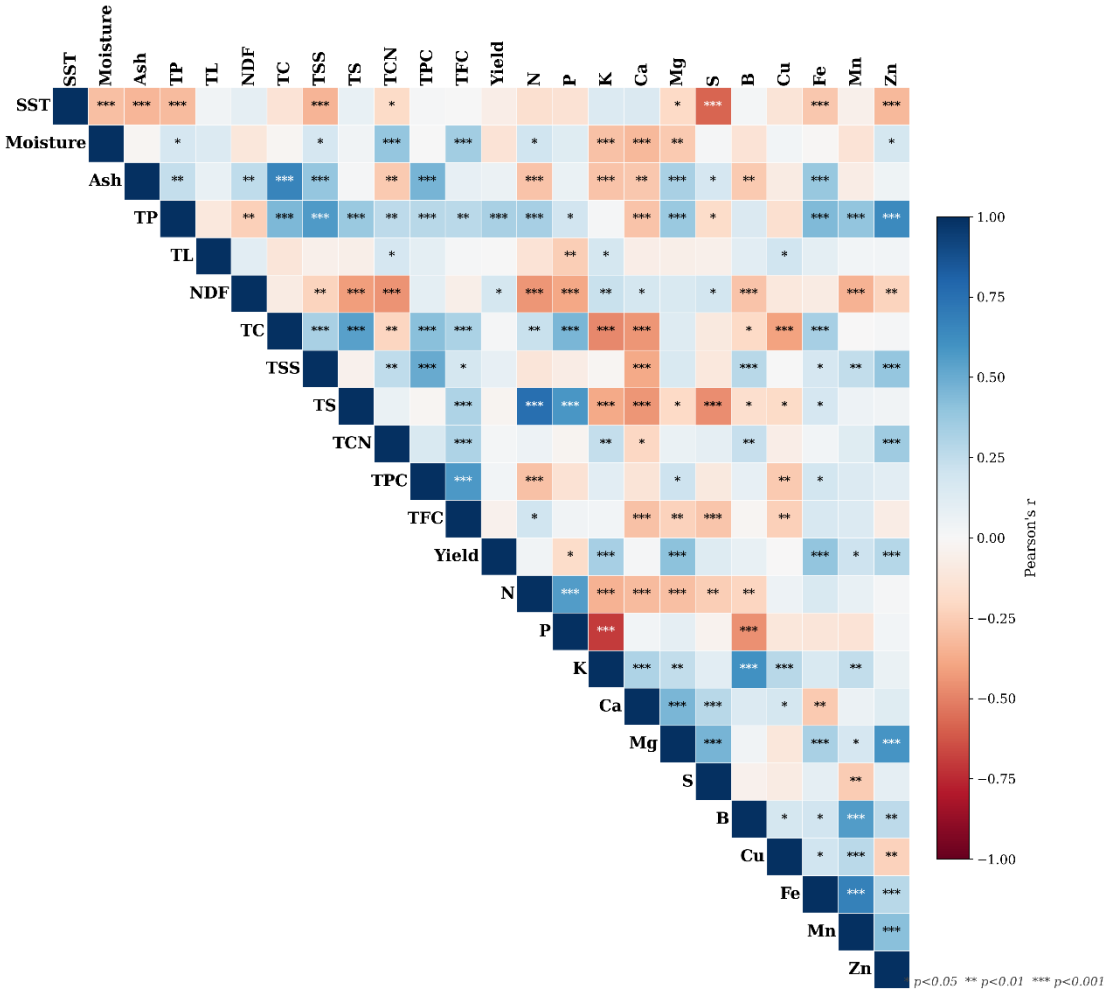


Figure 9 - Pearson correlation matrix among sea surface temperature (SST), biochemical variables, and macro- and micronutrient contents of the industrial solid residue of Kappaphycus alvarezii obtained after aqueous extract production, cultivated in Palhoça and Florianópolis, Santa Catarina, Brazil (n = 48). Only the upper triangle is shown. Cell color indicates the direction and magnitude of the correlation coefficient (r), ranging from blue ($r = +1$, strong positive) to red ($r = −1$, strong negative). Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Cells without asterisks represent non-significant correlations ($p \geq 0.05$). Variable abbreviations: SST, sea surface temperature; Ash, ash content; TP, total protein; TL, total lipids; NDF, neutral detergent fiber; TC, total carbohydrates; TSS, total soluble sugars; TS, total starch; TCN, total carotenoids; TPC, total phenolic compounds; TFC, total flavonoid

compounds; Yield, carrageenan yield; N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; S, sulphur; B, boron; Cu, copper; Fe, iron; Mn, manganese; Zn, zinc.

3.7 Fatty acid profile (FA)

3.7.1 Saturated fatty acids (SFA)

Among the saturated fatty acids detected in the residue, heptadecanoic acid (C17:0) was the dominant compound across all sites and seasons, with relative area values ranging from 37.8% (PAL-7, Summer) to 80.2% (PAL-4, Winter). The highest concentrations were consistently recorded in Winter samples from PAL, with PAL-4 presenting the most extreme value in the entire dataset, suggesting a site- and season-specific accumulation of this odd-chain saturated acid in the residue during colder months at Palhoça. Lignoceric acid (C24:0) showed a markedly site-restricted distribution, being detected exclusively in PAL samples (6.8–21.7%), with complete absence from all RIB sites across every season, indicating a location-driven biosynthetic pattern independent of seasonality. This compound was most abundant in PAL-2 (Autumn, 21.7%) and PAL-6 (Spring, 16.8%), with no clear seasonal trend at PAL. Lauric acid (C12:0) was sporadic, detected at only six sites: PAL-1 (5.1%), PAL-7 (7.1%), PAL-8 (13.4%), RIB-2 (16.3%), RIB-8 (7.4%), with the highest value in RIB-2 (Autumn). Notably, C8:0 (caprylic acid), which was the fifth most abundant FA in the fresh biomass reported by Schneider et al. (2026) with an overall mean of 4.6%, was entirely absent from the residue, suggesting its preferential partitioning into the aqueous fraction during the extraction process.

3.7.2 Monounsaturated fatty acids (MUFA)

cis-10-Heptadecenoic acid (C17:1) was the most abundant MUFA in the residue and showed a pronounced location effect, being consistently detected at higher levels in RIB (3.3–16.1%) than in PAL, where it was absent from PAL-5 and PAL-6 (Spring) and near-trace in PAL-7 and PAL-8 (Summer, 0.6–0.7%). This pattern is fully consistent with the fresh biomass characterization of Schneider et al. (2026), who reported significantly higher C17:1 levels at RIB across all four seasons. Nervonic acid (C24:1n9) was present at both locations but with higher and more consistent values at RIB (2.2–12.2%) than at PAL (0–8.1%), where it was absent from PAL-4 and PAL-6. Myristoleic acid (C14:1) was detected at all sites except PAL-7, at relatively low and stable levels (1.3–4.9%), with no marked seasonal or location trend.

3.7.3 Polyunsaturated fatty acids (PUFA)

Linolelaidic acid (C18:2n6t) was the most abundant PUFA and the second most represented compound in the residue overall, with relative area values ranging from 10.3% (PAL-8) to 29.3% (RIB-6), and a tendency towards higher levels in Spring samples — particularly at RIB (RIB-5: 26.3%; RIB-6: 29.3%) — and lower levels in Winter at PAL (PAL-4: 10.3%). Linoleic acid (C18:2n6c) was present at all sites at low levels (0.8–9.4%), with a notable peak at RIB-2 (9.4%), which contrasts with the generally low values at the remaining sites (< 4%). cis-13,16-Docosadienoic acid (C22:2) showed a site-associated pattern with higher and more consistent values at RIB (2.6–7.1%) than at PAL, where it was absent from PAL-4, PAL-6, and PAL-7, and generally below 3% when present.

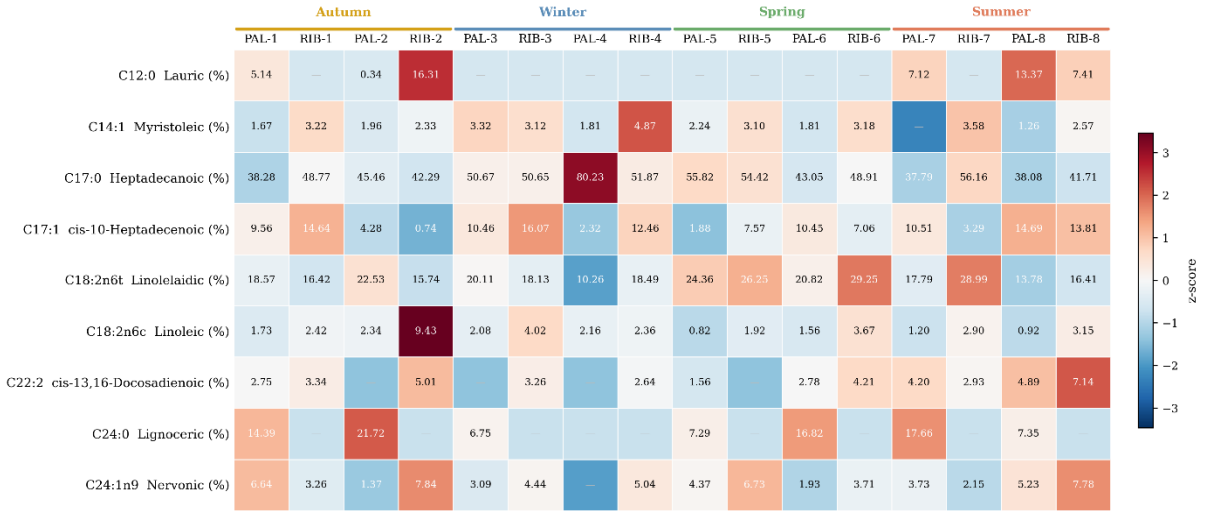


Figure 10 - Heatmap of the major fatty acid (FA) contents (% relative area) of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production, cultivated in Palhoça (PAL) and Florianópolis (RIB), Santa Catarina, Brazil. Only fatty acids detected in at least four of the 16 sampling sites are shown (n = 9). Rows represent individual fatty acids and columns represent sampling sites, ordered by season (Autumn, Winter, Spring, Summer) with PAL and RIB interleaved within each season. Cell colour indicates the relative abundance expressed as z-score per fatty acid, ranging from blue (high) to red (low). Numerical values represent the relative area of each fatty acid as a percentage of the total chromatogram area (100%). Fatty acid abbreviations: C12:0, lauric acid; C14:1, myristoleic acid; C17:0, heptadecanoic acid; C17:1, cis-10-heptadecenoic acid; C18:2n6t, linolelaidic acid; C18:2n6c, linoleic acid; C22:2, cis-13,16-docosadienoic acid; C24:0, lignoceric acid; C24:1n9, nervonic acid.

PCA of the nine FA compounds included in the heatmap yielded PC1 = 35.1%, PC2 = 23.8%, and PC3 = 15.8% (cumulative 74.7%). The score plot revealed a clear separation

between locations along PC2, with PAL samples displaced towards the positive region, principally driven by the strong loading of C24:0, and RIB samples shifted towards the negative region, associated with higher contributions of C14:1 and C18:2n6c (Figure 11). The exclusive presence of C24:0 at PAL reinforced this separation, acting as a discriminant variable between the two cultivation sites. Seasonal structuring was most evident among Winter samples: PAL-3 and PAL-4 were strongly isolated at the negative extreme of PC1, reflecting the exceptionally high C17:0 content of those samples, while RIB-3 and RIB-4 were positioned in an intermediate region. Spring samples clustered along the negative PC1 axis, consistent with elevated C17:0 and C14:1.

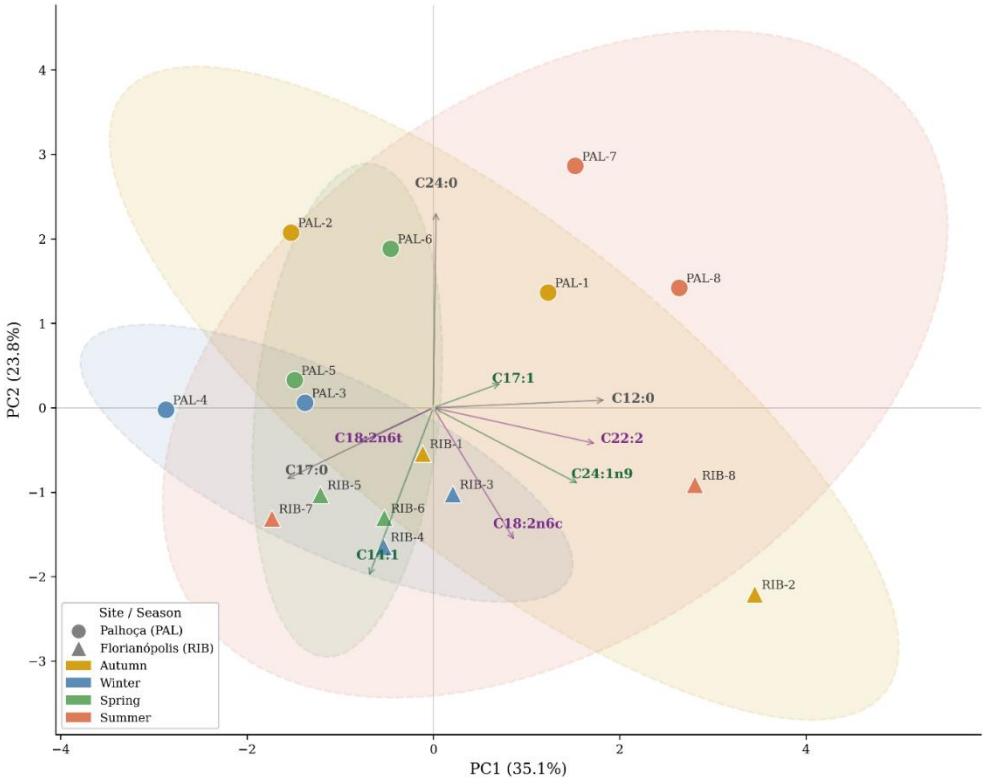


Figure 11 - Biplot of principal component analysis (PCA) of the major fatty acid profile of the industrial solid residue of Kappaphycus alvarezii obtained after aqueous extract production, cultivated in Palhoça (PAL) and Florianópolis (RIB), Santa Catarina, Brazil. Only fatty acids detected in at least eight of the 16 samples were retained for the analysis (C12:0, C14:1, C17:0, C17:1, C18:2n6t, C18:2n6c, C22:2, C24:1n9, C24:0 n = 9 variables). Variables were standardized to zero mean and unit variance prior to PCA. Scores (sites) are represented as circles (PAL) or triangles (RIB), colored by season. Loading vectors (arrows) indicate the direction and relative contribution of each fatty acid to the principal components. Dashed ellipses represent 95% confidence intervals for each seasonal group.

4. Discussion

The solid residue generated after ambient-temperature aqueous extraction of *K. alvarezii* retained a biochemically complex matrix, as evidenced by its high moisture (81.6–87.9%), elevated ash (25.4–31.9% dw), moderate lipids (mean 4.79%), low but detectable protein, substantial carbohydrates, residual carrageenan (mean 17.9%), and measurable bioactive compounds. Compared with the fresh biomass from the same cultivation sites [14], the residue exhibited selective retention of structural polysaccharides, minerals, lipids, and carrageenan, consistent with the non-thermal extraction process. The following discussion integrates these compositional patterns with seasonal and site-specific environmental drivers, then translates them into concrete opportunities for food ingredient development and cascading biorefinery.

4.1 Seasonal and Site-Specific Drivers of Residue Composition

The biochemical composition of the *K. alvarezii* solid residue varied markedly with season and, to a lesser extent, with cultivation site. These patterns are best understood through the two partially independent axes identified in the principal component analysis of biochemical variables: a seasonal axis (PC1, 27.8% of variance) governing carbohydrate and bioactive accumulation, and a spatial axis (PC2, 19.1%) reflecting constitutive differences in mineral content, moisture, and carotenoid accumulation between cultivation sites. Together, these two axes accounted for nearly half (46.9%) of total biochemical variance, confirming that the composition of the residue is systematically co-shaped by temporal and spatial environmental factors, a finding with direct implications for standardizing residue-derived products [33–37].

Along the seasonal axis (PC1), Autumn samples presented the highest mean scores (+1.47) and Spring the lowest (−1.52), consistent with the strong carbohydrate depletion observed in Spring at both locations. This depletion was most striking for total starch, whose Spring mean was three times lower than values recorded in Autumn, Winter, and Summer, indicating mobilization of reserve carbon to support growth as temperatures and photoperiod increase [13,38]. The coincidence of low starch with elevated NDF and sulphur (S) in Spring may reflect a seasonal reallocation of metabolic resources from storage polysaccharides towards cell wall synthesis and sulphated polysaccharide production (carrageenans), a pattern consistent with the positive correlation between SST and potassium (K), given potassium's established role as an osmolyte in seaweeds [39], and with the spring peak in sulphur content

(at PAL), possibly reflecting increased investment in sulphated polysaccharides [40]. However, further research is needed to determine whether the thermal conditions of spring upregulate the carrageenan biosynthesis enzymes [41,42].

The temporal patterns identified for individual variables were corroborated by Pearson correlation analysis, which revealed significant negative associations between SST and ash content, total soluble sugars, total protein, and moisture. These relationships indicate that warmer sea surface temperatures are associated with systematic reductions in mineral content, soluble sugars, protein, and water retention in the residue, providing a quantitative mechanistic link between the environmental thermal gradient, which oscillates between approximately 17°C in Winter and 26°C in Summer at the study sites, and the biochemical state of the co-product. SST was not significantly correlated with total lipids, NDF, total carbohydrates, total starch, total carotenoids, total phenolics, total flavonoids, carrageenan yield, or the micronutrients boron, copper, iron, manganese, and zinc, suggesting that these fractions are regulated by factors beyond sea surface temperature alone, such as photoperiod, nutrient availability, or site-specific hydrodynamics.

Along the spatial axis (PC2), RIB samples scored positively, principally driven by higher carotenoid content, moisture, and total lipids, while PAL samples scored negatively, reflecting higher and more stable ash and mineral contents. The divergent behavior of phenolic compounds between sites (PAL showing peak values in Summer and RIB lowest values in Spring and Summer) is consistent with the modulation of phenolic synthesis by multiple environmental stressors (irradiance, temperature, nutrient availability, hydrodynamics) rather than by temperature alone [43–46]. The elevated TPC at PAL during Summer and Autumn may reflect a combined response to higher UV radiation and thermal stress, while lower values at RIB suggest that site-specific factors such as water depth, turbidity, or local hydrodynamics, modulate the TPC response independently of temperature. It should be noted that, because nutrient values represent single measurements per site replicated across three biochemical subsamples, correlations involving nutrients may be subject to a degree of inflation relative to those computed entirely from replicated data, and should be interpreted with corresponding caution.

4.2 Proximate composition and extraction efficiency

Moisture remained high (81.6–87.9%), with only one significant seasonal within-site difference detected (RIB-Spring higher than PAL-Spring), indicating that a substantial portion of water is structurally bound to cell wall polysaccharides and not released by mechanical pressing. From a food processing perspective, this high moisture necessitates drying for any application as a functional ingredient (e.g., seaweed flour, fortified matrices). Drying reduces water activity, extends shelf life, prevents microbial spoilage and preserves bioactive compounds [19,47]. For the carrageenan industry, moisture above 35 % promotes degradation, and above 40 % can cause near-complete loss of functional properties [48], making drying an indispensable processing step.

In contrast to moisture, ash content showed a pronounced and consistent spatial pattern: PAL samples maintained high and relatively stable levels across all seasons, while RIB showed a progressive decline from Autumn through to Summer, a pattern confirmed by the strong positive loading of ash on the PAL-associated negative region of PC2. This divergence extended to the underlying mineral profiles. Potassium accumulated progressively in Spring and Summer at both sites, coinciding with its positive correlation with SST. Calcium and magnesium reached exceptionally high values in Spring, particularly at PAL. Sulphur also peaked in Spring, and the strong positive correlations between Ca, Mg, and S support their co-accumulation during a period characterized by active cell wall synthesis in red algae. Iron and manganese were highest in Winter, and their positive correlation is consistent with their shared geochemical behavior in marine environments. From a food application perspective, the high and seasonally stable ash content of PAL residue makes it a consistent natural mineral supplement for food fortification: e.g., in functional bakery products, pasta matrices, and nutraceutical blends, where Ca, Mg, and K can partially replace synthetic premixes. The elevated iron content in Winter samples suggests potential for addressing iron deficiency in cereal-based products or fortified beverages. The Spring-specific peaks in Ca, Mg, and K are well-suited for dairy alternatives, plant-based milks, and sports nutrition formulations. Site-specific mineral signatures (higher Ca and S at PAL, higher Fe and Mn at RIB) could additionally serve as biochemical traceability markers in quality control frameworks. The absence of detectable heavy metals in the aqueous extract from the same farms [7,14] implies that the residue is likely safe for food use, in alignment with ANVISA and EFSA limits, though direct heavy metal analysis of the residue is recommended to confirm this inference.

In relation to TP, it ranged from 0.40 to 1.09 mg g^{-1} (Bradford-reactive, extractable protein). These values are much lower than Kjeldahl-based crude protein estimates in whole biomass (12.7–23.6 g/100 g DW), a methodological distinction that should not be misinterpreted as a protein-poor matrix. The extractable fraction represents soluble and cell-wall-loosely-associated proteins; structural proteins (phycobiliproteins, glycoproteins) are only partially captured due to the complex polysaccharide cell wall [49,50]. Despite modest absolute values, the positive correlation between TP and TSS and between TP and TC may reflect a coupled anabolic state in which carbon and nitrogen resources are simultaneously mobilized during periods of active growth, with Winter RIB samples representing peak protein accumulation in the residue. Protein concentrates from *K. alvarezii* exhibit high water and oil absorption capacities and stable emulsifying activity [51], suggesting techno-functional value in emulsified meat products, plant-based alternatives or protein-enriched bakery formulations.

4.3 Carbohydrate fractions and neutral detergent fiber (NDF)

Total carbohydrates (TC) ranged from 238 to 537 mg g^{-1}, with spring samples showing the greatest depletion, consistent with reserve mobilization to support growth under increasing light and temperature [13,38]. The strong positive correlation between TC and ash content suggests that both variables are maximized under cooler, high-mineral-availability conditions during autumns and winter [52,53] and share a common physiological basis.

Total soluble sugars (TSS) were dramatically reduced in the residue (1.2–8.2 mg g^{-1}) compared to fresh biomass [14], confirming efficient transfer to the aqueous phase. In contrast, total starch (TS) was markedly enriched (78.7–266.2 mg g^{-1}) representing a substantial concentration effect as soluble fractions are removed. The strong positive correlation between TS and TC confirms that starch represents a major fraction of the total carbohydrate pool in this residue Floridean starch, a branched α-glucan characteristic of red algae, is readily hydrolyzed by α-amylase and can be enzymatically saccharified to glucose with high efficiency [8,9]. Winter and summer residues are therefore attractive substrates for enzymatic glucose production or as directly digestible carbohydrate in functional food matrices.

Within this carbohydrate pool, the neutral detergent fiber (NDF) fraction (9.2–33.2% DW) encompasses cellulose and residual insoluble polysaccharides, broadly equivalent to insoluble dietary fiber in food biochemistry. Its relevance as a functional food ingredient derives from three convergent properties: resistance to gastrointestinal digestion,

fermentability by colonic microbiota, and capacity to mimic fat texture in reduced-fat formulations. NDF was negatively loaded on PC2 in the biochemical PCA, associating its higher values with PAL samples, particularly in Spring. Regarding fermentability, Bajury et al. [54] demonstrated that digested *K. alvarezii* biomass fermented with human fecal inocula increased *Bifidobacterium* spp. populations (comparable to inulin) with elevated acetate and propionate production, key short-chain fatty acid mediators of colonic health. More broadly, the sulfated polysaccharides of red algal cell walls, including carrageenan, exhibit selective fermentability and immunomodulatory activities not shared by terrestrial plant fibers, supporting their potential as prebiotic ingredients for gut health modulation [55]. However, the prebiotic potential of carrageenan must be weighed against safety concerns raised by pre-clinical evidence. Low-molecular-weight carrageenan oligosaccharides have been shown to stimulate pro-inflammatory cytokine secretion (IL-1β, TNF-α) in human colonic cells, and κ-, ι-, and λ-carrageenan isoforms induced colitis and suppressed *Akkermansia muciniphila* in murine models [56]. These effects appear most pronounced for degraded, low-molecular-weight forms administered as isolated food additives; the residue described here retains carrageenan as part of an intact, high-molecular-weight cell wall matrix, which may confer a distinct physiological behavior. Nevertheless, the suitability of this residue for direct human consumption warrants dedicated safety and fermentability studies prior to food formulation [56]. Importantly, these safety considerations pertain primarily to carrageenan consumed chronically as an isolated food additive, and do not preclude its recovery from the residue as a purified ingredient for industrial food applications, a valorization pathway addressed in the following section.

4.4 Carrageenan yield

A particularly valuable component of the insoluble carbohydrate fraction is residual carrageenan. The residual carrageenan yields (12.3–23% DW) are comparable to commercial yields from fresh biomass under optimized alkaline extraction (20–49% DW) [8,25]. Within this range, PAL-Autumn presented the lowest yield and was significantly lower than both PAL-Winter and RIB-Winter, with PAL-Autumn also recording a significantly lower value than RIB-Autumn, the only season in which a significant between-site difference was detected. Winter samples yielded the highest retention at both sites, indicating that seasonal scheduling can maximize carrageenan recovery. This is consistent with patterns documented for Indian *K. alvarezii* populations, where carrageenan quality and yield peak in cooler months [12], and is

also supported by the positive loading of carrageenan yield on the cooler, mineral-rich end of PC1 in the biochemical PCA.

Co-production of biostimulant extract and carrageenan from *K. alvarezii* is economically superior to single-product extraction [57]; the present study provides seasonal yield data to extend this concept to the residue. In food systems, κ-carrageenan functions as a gelling, thickening and stabilizing agent in dairy products, processed meats, plant-based foods and infant formula [4]. Carrageenan recovered from this type of mechanical-press solid residue may retain higher-molecular-weight fractions, since the ambient-temperature aqueous extraction selectively partitions low-molecular-weight soluble compounds into the liquid phase; given the established positive relationship between molecular weight and gel strength in *K. alvarezii* carrageenan [58], this selective retention could translate into superior gelling performance relative to conventionally extracted preparations. This hypothesis, however, warrants direct rheological characterization of residue-derived carrageenan. On this basis, carrageenan recovery should be the first valorization step, with the carrageenan-depleted fraction entering secondary streams.

4.5 Bioactive compounds: phenolics, flavonoids and carotenoids

In addition to structural and storage polysaccharides, the residue retains a suite of low-molecular-weight bioactive compounds measurable across all sampling groups: total phenolic compounds (TPC: 17–67 mg 100 g^{-1}), total flavonoids (TFC: 3.2–13.5 mg 100 g^{-1}), and total carotenoids (TCN: 72–249 mg 100 g^{-1}). Comparison with the fresh biomass from the same farms and sampling period [14] reveals markedly distinct partition dynamics for each compound class across the extraction process. TPC in the fresh biomass ranged from 20.2 to 212.5 mg 100 g^{-1}, with spring and summer seasonal means of 113.7 and 100.6 mg 100 g^{-1}, respectively, substantially exceeding the residue's maximum of 67 mg 100 g^{-1}. This indicates that part of soluble phenolics are transferred to the aqueous extract phase, with the residue retaining primarily cell wall-bound or sparingly water-soluble fractions [59]. This pre-concentration of bound phenolics makes the residue an efficient starting material for hydroalcoholic extraction, requiring lower solvent volumes than fresh biomass.

In contrast to TPC, TFC showed a markedly different behavior: fresh biomass values (4.2–16.9 mg 100 g^{-1}) [14] are broadly comparable to those found in the residue (3.2–13.5 mg 100 g^{-1}), suggesting that flavonoids (as a subclass of phenolics) are largely retained in the solid

co-product rather than transferred to the aqueous phase, consistent with their generally lower aqueous solubility [60]. TPC and TFC were highest at PAL in Autumn and Summer, while RIB Spring and Summer showed the lowest TPC values in the dataset. These patterns align partially with Nunes et al. [7], who characterized lab-scale residues from the same RIB farm in summer alongside a São Paulo farm and found that SC-origin residues generally maintained higher TPC than SP-origin residues. Seaweed polyphenols have documented antioxidant, anti-inflammatory, antimicrobial, and anticancer properties [59]. Their incorporation into functional foods and nutraceuticals [61] (Baghel et al., 2023), and their application in active packaging systems [62] have been extensively reviewed, supporting the sustainable valorization of this residue.

Total carotenoid content (TCN) showed the most striking reduction across the extraction process: fresh biomass TCN ranged from 355 to 1242.7 mg 100 g^{-1} [14], while residue values reached only 72–249 mg 100 g^{-1}, a reduction of approximately 70–90%, indicating that most carotenoids are either transferred to the aqueous extract or degraded during ambient-temperature pressing. Despite this reduction, residue TCN remained measurable and spatially structured: RIB Winter samples (248.96 mg 100 g^{-1}) recorded more than three times the carotenoid content of PAL Winter (72.08 mg 100 g^{-1}), a between-site pattern not evident in the fresh biomass [14]. The positive correlation between TCN and moisture and its negative correlation with NDF suggest that carotenoid retention in the residue is favored in samples with higher water content and reduced structural fiber density. The concentrations retained in Winter and Spring RIB residues identify these fractions as viable substrates for carotenoid recovery using food-grade solvents as a third-stage valorization stream.

Carotenoids are among the most commercially valuable natural pigments. In the food industry, β-carotene and α-carotene, both prevalent in red algae [63] are approved natural colorants applied across a wide range of matrices including dairy products, fruit juices, baked goods, confectionery, processed meats, and surimi, where they provide yellow-to-orange hues as clean-label alternatives to synthetic dyes [64]. Zeaxanthin, also reported in *K. alvarezii*, has documented associations with macular degeneration prevention and is increasingly incorporated into functional foods and dietary supplements targeting eye health [59].

4.6 Total lipids and fatty acid profile

Total lipid content (mean 4.79% DW) was quantitatively retained in the residue, falling within the upper range reported for fresh biomass (1.5-5.1% DW) [14], and the absence of significant seasonal or location effects suggests constitutive regulation of lipid accumulation independent of the environmental gradients that drive carbohydrate and bioactive variability. At this concentration, the lipid fraction is not sufficient to position the residue as a primary lipid source in food formulations; rather, its value lies in the quality of the fatty acid profile it delivers as part of a complex multicomponent matrix. In this context, the most well-supported application is the incorporation of the dried residue as a functional ingredient in aquaculture and animal feeds, where the combination of lipids, specific fatty acids, carrageenan-derived fiber, minerals, and bioactive compounds at 3–10% dietary inclusion has been documented to improve growth performance, immune response, and oxidative stability in finfish and crustaceans [65,66]. The constitutive retention of lipids across seasons and sites also confers formulation consistency, a relevant advantage in feed manufacturing, where ingredient variability is a primary challenge.

The fatty acid (FA) profile of the residue was dominated by heptadecanoic acid (C17:0; 37.8–80.2% of total area), consistent with previous characterizations of *K. alvarezii* [1,14]. Circulating odd-chain saturated FA, including C17:0, are inversely associated with incident type 2 diabetes [67] and are proposed to influence metabolic regulation [68]. The exceptionally high C17:0 content in winter samples from PAL (up to 80.2%) suggests potential for nutraceutical ingredient development, though absolute quantification per gram of dry residue is needed. Lignoceric acid (C24:0), another saturated FA, was detected exclusively in PAL samples (6.8–21.7%), representing a striking site-specific signature. C24:0 is a major constituent of ceramides in the human stratum corneum, essential for skin barrier function [69]. Nervonic acid (C24:1n9) was higher in RIB samples (up to 12.2%), consistent with the fresh biomass [14], and is essential for myelination, with proposed therapeutic potential for demyelinating conditions [69]. The co-presence of C24:0 and C24:1n9 in the residue, enriched relative to fresh biomass, positions PAL-derived residue as a potential substrate for long-chain fatty acid extraction targeting cosmeceutical (skin barrier) and neuroprotective nutraceutical applications. It is important to note that the contrasting fatty acid signatures between sites, C24:0 exclusive to PAL, C17:1 and C22:2 higher at RIB, confirmed by PCA, support the use of fatty acid profiles as molecular traceability markers for origin authentication.

Among polyunsaturated fatty acids, linolelaidic acid (C18:2n6t) was the most abundant, with higher relative areas in spring samples, particularly at RIB (up to 29.3%), consistent with the fresh biomass [1,14]. Linoleic acid (C18:2n6c), an essential omega-6 fatty acid for mammals, was present at low but consistent levels (0.8–9.4%). It should be noted that linolelaidic acid is a trans isomer whose biological effects differ from its cis counterpart, and its specific implications for seaweed-derived ingredients require further study. From a food industry perspective, the presence of essential linoleic acid contributes to the nutritional value of the residue for human [70] and animal nutrition [71,72].

4.7. Cascading valorization within a blue bioeconomy framework

The comprehensive biochemical dataset presented in this study provides the quantitative foundation for a cascading valorization model of the *K. alvarezii* post-aqueous extract residue. Cascading biorefinery approaches, in which each processing step generates a primary high-value product and a secondary substrate for the next stage, have been demonstrated at pilot scale for *Saccharina latissima* [73] and *Sargassum muticum* [74]. This concept is also well-established for red seaweeds. A review by Torres et al. [75] proposes the sequential separation and valorization of red seaweed components for food, nutraceutical, cosmeceutical and pharmaceutical applications, highlighting that carrageenan is the major fraction commercially extracted. Álvarez-Viñas et al. [76] present practical successful examples of red seaweed biorefineries, where cascading stages of conventional and alternative techniques ensure full resource utilization and generate few residues. Hans et al. [77], using supercritical fluid extraction (SFE) on *K. alvarezii* biomass, obtained a lipid yield of 0.21 ± 0.2 %, rich in polyunsaturated fatty acids (9.12 %), and subsequently recovered ~56 % of κ-carrageenan from the SFE-treated residue. Similarly, Rudke et al. [78] investigated eco-friendly methods for the sequential recovery of fatty acids, phenolics, and carrageenan from *K. alvarezii*. The concept also extends to other red seaweed processing residues: the solid by-product of agar extraction from *Gelidium corneum* was shown to contain high-value lipids, including omega-3 and omega-6 PUFAs, with potential applications in foods and cosmetics [79]. Likewise, ultrasound-assisted extraction enabled the enrichment of lipids from agar production wastes of *Gracilaria lemaneiformis*, yielding 0.71 % lipids enriched in unsaturated fatty acids [80]. Moreover, a sequential biorefinery approach applied to the carrageenophyte *Gigartina pistillata* extracted lipids and carotenoids by SFE, followed by carrageenan recovery from the residual solids, with the carrageenan exhibiting enhanced gel properties [81]. These

studies strongly support the industrial viability and sustainability of the cascading biorefinery model proposed here for the *K. alvarezii* residue.

For the *K. alvarezii* post aqueous extract residue, the proposed cascade begins with carrageenan recovery as the first and highest-value step. The carrageenan-depleted solid then enters the second-stage extraction: lipids for fatty acid recovery, followed by carotenoid extraction from winter/spring RIB residue, and phenolic/flavonoid enrichment from autumn/summer PAL residue. Starch in the carbohydrate-rich autumn fraction can be enzymatically hydrolyzed to glucose for fermentation or used directly as a functional ingredient in gluten-free food formulations [8]. The final mineral-rich organic fraction, after lipid, pigment and polysaccharide extraction, can be valorized in agriculture as a slow-release mineral biofertilizer, providing N, K, Ca, Mg, Fe and Zn to crops [82,83].

The seasonal scheduling of this cascade is the central practical insight of the present study: winter and autumn biomass should be directed preferentially towards carrageenan and starch extraction; winter RIB residue towards fatty acid and carotenoid recovery; and summer and autumn PAL residue towards phenolic and flavonoid concentrates. This season-optimized approach directly addresses the challenge of seasonal compositional variability, transforming it from an obstacle to product standardization into a competitive advantage that enables a diverse, year-round portfolio of speciality co-products from a single biomass source. Given the expanding cultivation of *K. alvarezii* along the Brazilian coast and the existing infrastructure for biostimulant production, the proposed season-optimized cascading biorefinery offers a practical and economically attractive pathway to fully harness the biochemical value of the residue, aligning with the principles of a circular blue bioeconomy and supporting the sustainable growth of the national seaweed industry.

Conclusions

This study provides the first seasonally resolved, farm-specific biochemical characterization of the solid residue generated during industrial aqueous extract production of *K. alvarezii* in southern Brazil. The residue retained a complex matrix of structural polysaccharides, minerals, lipids, residual carrageenan, bioactive compounds (phenolics, flavonoids and carotenoids), and a fatty acid profile dominated by heptadecanoic acid (C17:0). Season and cultivation site systematically shaped its composition: winter samples were richest in carrageenan, starch, Fe, and Mn; spring samples in Ca, Mg, K, and S; summer/autumn

residues from PAL in phenolics and flavonoids; and winter/spring residues from RIB in carotenoids. The residue is suitable for food fortification, prebiotic dietary fiber (NDF), natural colorants (carotenoids), and gelling/thickening applications (carrageenan). A cascading biorefinery model is proposed: carrageenan recovery first, followed by sequential extraction of lipids, carotenoids, phenolics, and starch, with the final mineral-rich fraction used as a slow-release biofertilizer. Seasonal scheduling (winter for carrageenan/starch, spring/summer for bioactive compounds) turns compositional variability into a competitive advantage, supporting a circular blue bioeconomy for Brazil's expanding seaweed sector.

Acknowledgment

To the companies that provided the *Kappaphycus alvarezii* biomass for analysis. Centro De Desenvolvimento Em Aquicultura E Pesca - Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina. The research fellowship from CNPq (process no. 405949/2022-7) on behalf of M. Maraschin and on behalf of Giusepina Lima (process no. 307571/2019-0) from National Council for Scientific and Technological Development, Brazil (CNPq). The research fellowship from CNPq on behalf of A.R. Schneider (process no. 142391/2020-4) and E.R.O. Rodrigues (process no. 303956/2023-2). The research fellowship from CAPES on behalf of F.S.D. (process no. 88887.696139/2022-00). Grant 2023/03886-1, São Paulo Research Foundation (FAPESP), on behalf of A. Nunes.

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Capítulo 3 – *Priming* em sementes de cevada (*Hordeum vulgare* L.)

Seed priming with *Kappaphycus alvarezii* biostimulant elicits hormetic growth and metabolic reprogramming in barley seedlings

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Abstract

Seed priming with biostimulants derived from macroalgae represents a promising strategy for enhancing seedling performance in sustainable cereal production, yet the dose-response relationships governing these effects remain poorly characterized. This study evaluated the morphophysiological and biochemical responses of barley (*Hordeum vulgare*) seedlings to seed priming with *Kappaphycus alvarezii* biostimulant across seven concentrations (0, 0.5, 1, 2, 5, 10, and 20% v/v), modeled using the Brain-Cousens four-parameter hormesis equation. Priming at 2–5% maximized shoot and root biomass, seedling elongation, and the Seedling Vigor Index, with shoot fresh mass peaking at 5% (+35% over the control) and root fresh mass reaching a maximum at 0.5% (+41%). The contents of chlorophylls *a* and *b*, total chlorophyll, and carotenoids peaked at 2% (~+20%), following a unimodal rather than hormetic profile. A concentration-dependent metabolic reprogramming was identified: total starch declined monotonically by 59% between the control and 10%-treated seeds, while total protein increased non-monotonically, and the starch:protein ratio fell from 6.31 ± 0.28 to 1.95 ± 0.50, reflecting a progressive shift from carbon storage to protein anabolic metabolism. Multivariate analyses (principal component analysis and Z-score heatmaps) confirmed that a concentration of 2–5% was ideal for almost all variables evaluated. These results establish *K. alvarezii* biostimulant as an effective, multi-target seed-priming agent for barley, with a well-defined optimal concentration range and mechanistic evidence linking the extract's bioactivity to pre-germinative metabolic reprogramming.

Keywords: dose-response; hormesis; *Hordeum vulgare*; seedling vigor; starch mobilization, seaweed.

1. Introduction

Intensifying pressure on global food systems, driven by population growth, soil degradation, and the documented environmental externalities of synthetic agrochemical overuse, has accelerated demand for biologically derived crop inputs [1]. Among these, seaweed-based biofertilizers and biostimulants have attracted scientific and commercial attention owing to their complex composition of bioactive molecules that can modulate plant physiology throughout the crop cycle, from seed germination to post-harvest quality [2]. Phytohormones, structural polysaccharides, and mineral micronutrients are among the constituents that underpin the growth-stimulatory and stress-tolerance-enhancing properties attributed to macroalgal extracts [2,3], making them appealing alternatives to conventional chemical inputs.

Kappaphycus alvarezii (Doty) L.M.Liao (Rhodophyta) ranks among the most globally cultivated macroalgae, a position largely underpinned by the commercial value of its principal structural polysaccharide, kappa-carrageenan [4]. Beyond the hydrocolloid industry, this tropical red alga has emerged as a source of agriculturally relevant bioactive compounds, including endogenous plant growth regulators, defense-activating molecules, and soluble potassium [5–7], which together explain the agronomic improvements recorded across a broad spectrum of crops, from sugarcane and soybean to key cereals including maize, rice, and wheat [8]. In this context, preliminary foliar application studies with K. alvarezii extract on barley have already indicated positive agronomic responses [9]. Although these improvements have been achieved predominantly through foliar sprays and soil drenches, a growing body of evidence indicates that the same bioactive constituents can be effectively delivered via seed priming, targeting the very onset of the plant life cycle.

Seed priming is a pre-sowing technique in which seeds undergo controlled hydration in water or a priming solution for a defined period, followed by desiccation to near-original moisture content before germination irreversibly initiates [10]. This controlled imbibition activates pre-germinative metabolism, including cellular respiration, antioxidant pathways, and DNA repair, resulting in faster, more uniform germination and enhanced seedling vigor, even under adverse conditions [10]. The growing interest in ecologically sound priming agents has

driven research into the use of seaweed extracts as biostimulant seed treatments [11,12]. The extracts of the green alga *Ulva lactuca*, for example, improved germination percentage and seedling growth and enhanced antioxidant enzymatic activity in *Citrullus lanatus* seeds germinated under salt stress [12]. Similarly, the extract of *K. alvarezii*, used as a seed-priming agent in *Capsicum frutescens*, significantly improved germination percentage, mean germination time, vigor index, and seedling weight, as well as physiological and biochemical traits of primed seeds [11]. More recently, seed priming with K-sap in combination with foliar application was shown to enhance shoot and root growth, chlorophyll content, yield-attributing traits, and seed yield by up to 30% in mung bean (*Vigna radiata* L.) [13], confirming the efficacy of *K. alvarezii* extracts across diverse legume and cereal crops.

Nevertheless, the relationship between biostimulant concentration and plant response during seed priming is rarely addressed through rigorous quantitative dose-response modeling. Many biostimulants, including seaweed extracts, exhibit biphasic (hormetic) dose-response dynamics, in which low concentrations stimulate while high concentrations may inhibit growth. This nuance is lost when testing only a narrow concentration range without model fitting [14,15]. The Brain-Cousens model [16] is specifically designed to capture this hormetic profile, allowing the estimation of the effective stimulatory and inhibitory concentrations across the dose spectrum. Its application to seed priming bioassays with seaweed extracts represents a methodologically rigorous approach to determining optimal application rates and understanding the continuum between stimulation and phytotoxicity.

However, despite the extensive use of *K. alvarezii* extract in other cereals and the documented efficacy of seaweed-based seed priming for other crops, its dose-dependent effects on barley seed performance and the early physiological and biochemical responses of seedlings have not yet been investigated. Barley ranks as a top-tier global cereal crop, whose harvested grain is predominantly allocated to food, feed, and industrial malting purposes [17]. Seedling establishment quality, defined by the speed, uniformity, and synchrony of germination and early growth, directly affects yield potential, input use efficiency, and stand uniformity [18].

Thus, this study aimed to evaluate the dose-dependent effects of *K. alvarezii* biostimulant on germination and early seedling development of barley (*H. vulgare* cv. Rubi). Seeds were primed with seven concentrations (0–20% v/v), and responses were modeled using the Brain-Cousens four-parameter hormesis equation, an approach not previously applied, to our knowledge, to seed priming with seaweed extracts, across morpho-physiological endpoints and primary metabolite and photosynthetic pigment profiles.

2. Materials and Methods

2.1. Biostimulant source and biochemical composition

The *K. alvarezii* biostimulant was kindly provided by VerdeMar Ltda., a marine farm in Palhoça, Santa Catarina state, southern Brazil, and corresponded to the same biomass batch (autumn-harvested) used in a previously reported foliar-application study in barley [9]. The biostimulant was obtained by mechanical processing of fresh biomass cultivated locally and was used as the 100% stock solution. Its biochemical and nutritional profile (Table 1) is therefore identical to that previously reported.

Table 1 – Biochemical and mineral composition of *Kappaphycus alvarezii* biostimulant used for barley seeds priming.

Biochemical and nutrient analysis	Mean ± SD
Total solid content (%)	3.85 ± 0.18
pH	6.57 ± 0.01
Conductivity (mS.cm^{-1})	60.56 ± 14.45
Total carbohydrate (mg g^{-1})	4.96 ± 1.60
Total soluble sugar (mg g^{-1})	1.79 ± 0.76
Total starch (mg g^{-1})	5.51 ± 1.17
Total phenolic content (mg 100 g^{-1})	16.78 ± 5.69
Total protein (mg 100 g^{-1})	29.38 ± 16.65
Total amino acid (mg 100 g^{-1})	3.23 ± 1.61
Total lipid (%)	3.73 ± 1.21
Organic carbon (%)	0.80 ± 0.91
Nitrogen (%)	ND
Phosphorus (%)	0.01 ± 0.00
Potassium (%)	1.62 ± 0.29
Calcium (%)	0.01 ± 0.00
Magnesium (%)	0.03 ± 0.01
Sulfur (%)	0.05 ± 0.02
Boron (mg Kg^{-1})	1.18 ± 0.55
Copper (mg Kg^{-1})	0.08 ± 0.04
Iron (mg Kg^{-1})	5.29 ± 2.11
Manganese (mg Kg^{-1})	0.39 ± 0.36
Sodium (mg Kg^{-1})	2470.40 ± 845.84
Zinc (mg Kg^{-1})	0.59 ± 0.31

Values represent mean ± standard deviation (n = 3). ND = not detected. Source: Dutra et al. [9]

2.2. Plant material and seed disinfection

Barley seeds (*Hordeum vulgare* L. cv. Rubi) were supplied by Ambev S.A. (Companhia de Bebidas das Américas) and exhibited a germination rate of 95–98% in preliminary tests. Before any treatment, seeds were surface-disinfected with 2% (w/v) sodium hypochlorite for 6 min, rinsed six times in distilled water, and oven-dried at 30°C with forced-air circulation overnight [19]. This procedure aimed to eliminate potential surface pathogens without affecting viability.

2.3. Seed priming and treatment design

The biostimulant was evaluated at seven concentrations, 0 (control), 0.5, 1, 2, 5, 10, and 20% (v/v). Solutions were prepared by diluting the extract (100%) in distilled water. Based on the total solid content of the stock solution (3.85 ± 0.18%, Table 1), the corresponding solid contents of the priming solutions were 0, 0.019, 0.039, 0.077, 0.193, 0.385, and 0.770%, respectively. For each treatment, 20 g of barley seeds were placed in a 50 mL Falcon tube, submerged in 40 mL of the corresponding biostimulant (2:1 v/w solution-to-seed ratio. Seeds were imbibed for 12 h (room temperature), then air-dried for 24 h under ambient conditions to remove excess surface moisture and stabilize their water content.

Primed seeds were sown on Germitest paper rolls moistened with the corresponding priming solution at a 2.5:1 (water:paper, w/w) ratio, following the protocols of Brasil [19]. Four rolls of 32 seeds each (n = 128 per treatment) were placed in a BOD incubator at 25 ± 1 °C under a 12h photoperiod. Germination percentage was scored on day 8. From each roll, 20 seedlings were randomly sampled for morphological measurements, while the remaining seedlings per treatment were pooled to form a composite shoot sample for biochemical analyses (n = 3).

2.4. Morphological and physiological analyses

The seedlings (n = 20 per treatment) were scanned, and shoot and root lengths (cm) were determined with ImageJ (v. 1.53t). Shoot and root fresh and dry masses (mg) were recorded on an analytical balance (model AY220, Shimadzu, São Paulo, Brazil; precision

±0.0001 g). Dry masses (DM) of the shoot and root were obtained after oven-drying at 40 °C for 72 h. The root:shoot ratio (R:S) was calculated as fresh mass root/fresh mass shoot.

2.5. Chlorophylls and carotenoids

Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (TChl), and total carotenoids (TCN) were extracted from 100 mg of fresh shoot tissue. Samples were incubated in 7 mL of dimethyl sulfoxide (DMSO) at 65 °C for 2 h, filtered, and the extract made up to 10 mL [20]. Absorbance was read at 480, 649, and 665 nm in a spectrophotometer (Bel Spectro LGS53). Concentrations (μg mL^{-1}) were calculated using equations 1, 2, and 3 [21], and the final values were converted to mg g^{-1} dry weight.

$$Chl\ a\ =\ [12.19\ x\ (A_{665}) -\ 3.45\ x\ (A_{649})] \quad (1)$$

$$Chl\ b\ =\ [21.99\ x\ (A_{649}) -\ 5.32\ x\ (A_{665})] \quad (2)$$

$$TCN\ =\ \frac{[1{,}000\ x\ (A_{480}) -\ 2.14\ x\ (Chl\ a) -\ 70.16\ x\ (Chl\ b)]}{220} \quad (3)$$

2.6. Total soluble sugars and total starch

The contents of total soluble sugars (TSS) and total starch (TS) were determined using a method adapted from Umbreit and Burris [22]. Briefly, TSS were extracted by mixing 50 mg of fresh shoot sample with 2 mL of methanol–chloroform–water (12:5:3, v/v/v). After centrifugation (4000 rpm, 10 min, 25ºC), the supernatant was collected, and the pellet was re-extracted. The combined supernatants were phase-separated by adding 1 mL of chloroform and 1.5 mL of water. 1 mL of the aqueous phase was mixed with 2 mL of 0.2% (w/v) anthrone in concentrated H_2SO_4, vortexed, heated at 100 °C for 3 min, and cooled. Absorbance at 630 nm was measured in a spectrophotometer (SpectraMax 190 microplate reader). For quantification, a calibration curve was constructed using a glucose analytical standard (Sigma-Aldrich Ltda – MO/USA, 7.8–500 μg mL^{-1}, $y = 0.0018x$, $r^2 = 0.9917$).

Total starch (TS) was determined from the remaining pellet after two hydrolyses with 2 mL of 30% (v/v) perchloric acid, followed by centrifugation at 4000 rpm for 10 min at 25ºC. 1 mL aliquot of the supernatant was added to the anthrone reagent (0.2% w/v in H_2SO_4) and

incubated as described above for the reaction. Absorbance at 630 nm was measured in a spectrophotometer (SpectraMax 190 microplate reader). A starch analytical standard (Sigma-Aldrich Ltda – MO/USA, 1.9–500 μg mL^{-1}, y = 0.0016x, R^2 = 0.9916) served as a reference for the calculation of TS content. TSS and TS amounts were expressed as mg g^{-1} dry weight (DW).

2.7. Total amino acids

Total amino acids (TAA) were extracted following the method of Silveira and Furlong [23]. For that, a sample (100 mg) of fresh shoot tissue was ground (Omini® ultra-homogenizer) in 2 mL of distilled water, vortexed, and centrifuged at 4000 rpm for 10 min. A 1 mL aliquot of the supernatant was mixed with 3 mL of 0.2% (w/v) ninhydrin in sodium phosphate buffer (pH 7.0). The reaction was heated in a boiling water bath (100 °C) for 30 min, cooled to room temperature, and the absorbance was measured at 570 nm using a microplate reader (ThermoPlate® P-reader). A calibration curve was prepared with L-proline analytical standards (Sigma-Aldrich Ltda – MO/USA, 1.95–500 μg mL^{-1}, y = 0.0018x, R^2 = 0.9962). Results were expressed as mg of proline equivalents per gram of dry weight (mg g^{-1} DW).

2.8. Total protein

Total protein (TP) content was determined using the Bradford method [24]. Fresh shoot tissue (200 mg) was homogenized in 10 mL of phosphate-buffered saline (PBS, pH 7.0), vortexed, and left to stand for 15 min. The homogenate was then centrifuged at 4000 rpm for 10 min. An aliquot (100 μL) of the supernatant was mixed with 5 mL of a 1:5 (v/v) dilution of Bradford reagent in distilled water and allowed to react for 5 min. Absorbance was measured at 595 nm using a microplate reader (ThermoPlate® P-reader). A calibration curve was prepared with bovine serum albumin (BSA) analytical standards (Sigma-Aldrich Ltda – MO/USA, 3.9–250 μg mL^{-1}, y = 0.0032x, R^2 = 0.9772). Results were expressed as mg of BSA equivalents per gram of dry weight (mg g^{-1} DW).

2.9. Dose-response modelling

The relationship between biostimulant concentration and each response variable was modeled with the Brain-Cousens four-parameter log-logistic function [16], implemented via the *drc* package [25]. The model uses equation 4.

$$f(x) = \frac{d + (f \times x)}{1 + b^{(\ln x - \ln e)}} \quad 4$$

Where x is the extract concentration (%), b is the slope at the inflection point, d is the upper asymptote, e is the inflection-point (reference) dose, and f is the hormesis magnitude parameter (a positive f indicates stimulation above d at low doses). Goodness-of-fit was assessed by the coefficient of determination (R^2), calculated from the residual and total sums of squares.

2.10. Statistical analysis

For each variable, normality and homoscedasticity were checked using the Shapiro-Wilk and Levene tests, respectively. When both assumptions were met, one-way ANOVA ($\alpha = 0.05$) followed by Tukey's HSD post-hoc test was applied; otherwise, the Kruskal-Wallis test with Dunn-Bonferroni correction was used. All statistical and graphical analyses were performed using the R packages (version 4.6.0): *readxl*, *dplyr*, *tidyr*, *ggplot2*, *car*, *agricolae, FSA*, *multcompLetters*, *drc*, *pheatmap*, *FactoMineR*, and *factoextra*.

Treatment means of morpho-physiological and biochemical variables were separately standardized to Z-scores. A heatmap was built with the *pheatmap* R package [26] to visualize the relative variation of each variable across the seven concentrations, with positive and negative Z-scores indicating values above and below the overall mean, respectively. Additionally, principal component analysis (PCA) was performed independently on the two standardized mean matrices using the R package *FactoMineR* [27].

3. Results

3.1. Germination

Barley seed germination was generally high across all concentrations of *K. alvarezii* biostimulant, ranging from 84.3% (20%) to 95.3% (control) (Fig. 1). Although a Kruskal-Wallis test indicated a statistically significant effect of treatment on germination ($H = 14.484$, $p = 0.025$), post-hoc pairwise comparisons yielded no significant differences among any of the seven concentrations, suggesting that even the highest concentrations tested did not substantially impair seed viability or the completion of germination. Nevertheless, a numerical trend toward reduced germination at the highest concentrations (10 and 20%) was apparent, which, in conjunction with the pronounced effects on seedling elongation described below, suggests that inhibitory effects of the extract manifest primarily at the post-germinative developmental stage rather than during germination *stricto sensu*.

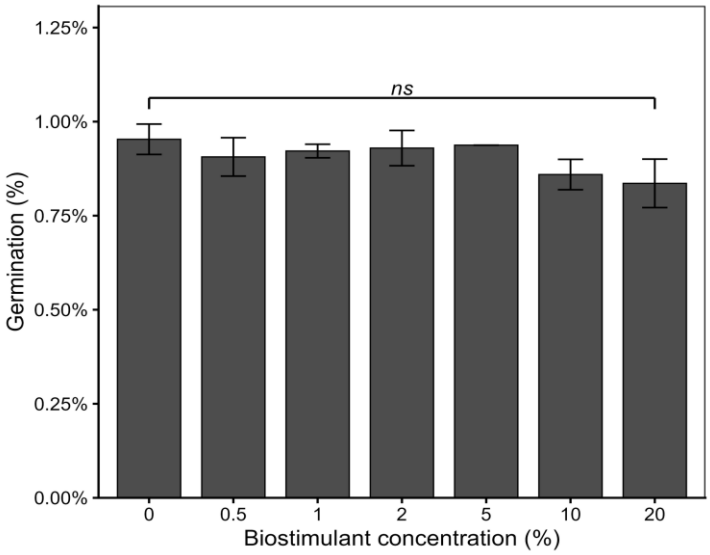

Fig. 1. Germination percentage of barley seeds after priming with *Kappaphycus alvarezii* biostimulant at seven concentrations (0, 0.5, 1, 2, 5, 10, and 20% v/v).

Bars represent means ± SE. ns = no significant, by Dunn-Bonferroni post-hoc test ($p>0.05$).

3.2. Morphophysiological variables

All morphophysiological variables assessed were significantly affected by seed priming concentration ($p < 0.001$ for all, except germination). Fresh and dry shoot mass exhibited significant concentration-dependent responses (ANOVA: $F = 11.418$ and $F = 6.380$, respectively; both $p < 0.001$). Fresh shoot mass (Fig. 3A) increased progressively from 110.96

± 18.45 mg in the control to a maximum of 149.88 ± 23.54 mg at 5% concentration, representing a 35.1% increase, before declining at 20% to values statistically equivalent to the control (114.86 ± 15.15 mg). This response was well-described by the BC.4 model ($R^2 = 0.999$), with a significant hormesis parameter ($f = 31.082 \pm 1.705$, $p < 0.001$) and an e-value of 5.971 ± 0.296% v/v, indicating that the stimulatory peak occurred near the 5–6% concentration range (Table 2). Dry shoot mass (Fig. 3B) followed the same pattern, peaking at 5% v/v (13.70 ± 2.22 mg) and reaching its minimum at 20% (10.20 ± 1.51 mg; BC.4: $R^2 = 0.904$, $f = 104.276 \pm 27.198$, $p = 0.031$).

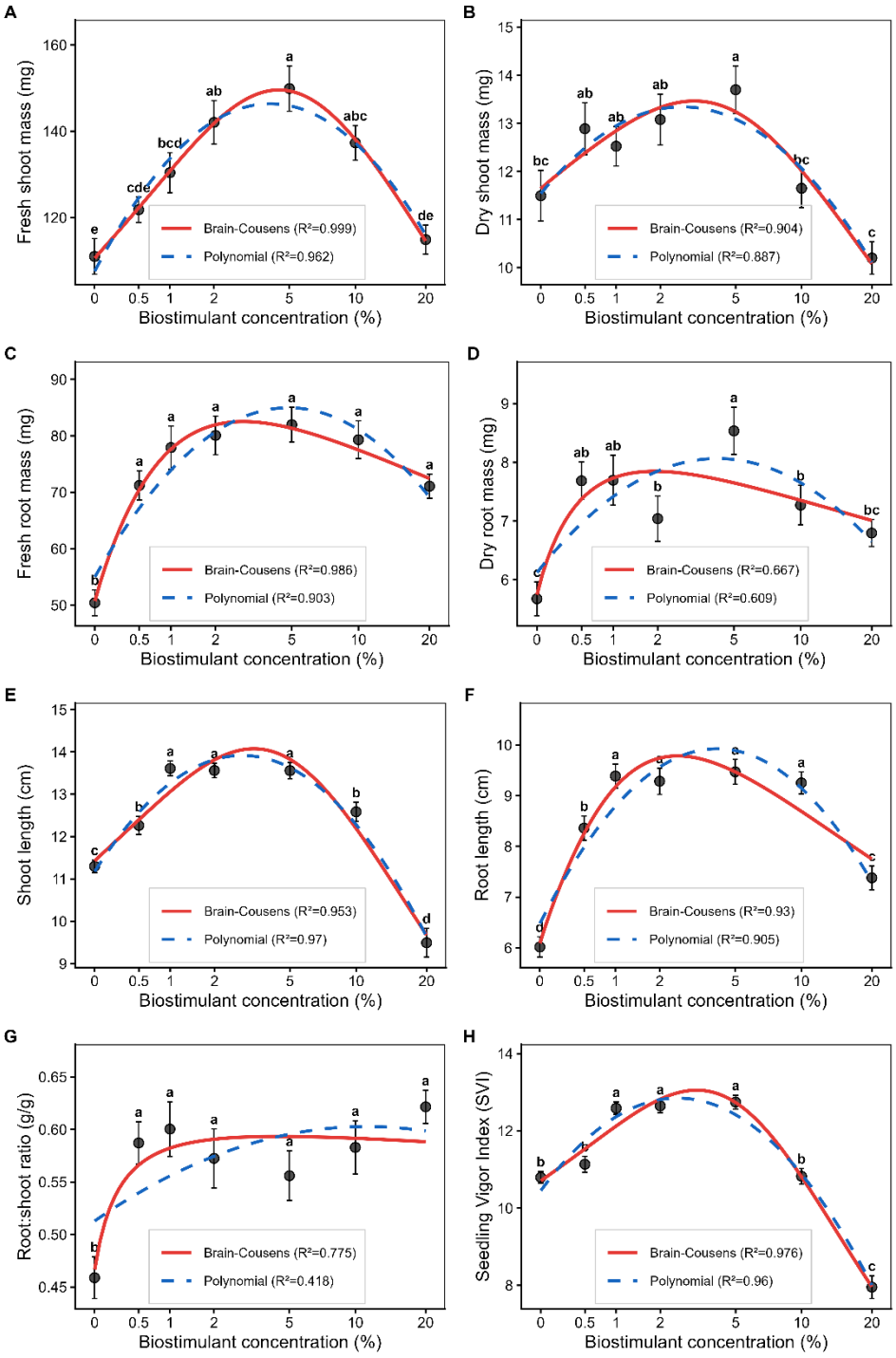

Fig. 2. Fresh shoot mass (A), dry shoot mass (B), fresh root mass (C), dry root mass (D), shoot length (E), root length (F), root:shoot ratio (G), and seedling vigor index (H) of barley seedlings to seed priming with *Kappaphycus alvarezii* biostimulant at seven concentrations (0, 0.5, 1, 2, 5, 10, and 20% v/v).

The x-axis is plotted on a log(dose + 1) scale. Red curves represent Brain-Cousens four-parameter and blue-dashed curves represent polynomial regression dose-response model fits. Different letters indicate significant differences among treatments (Tukey HSD or Dunn-Bonferroni post hoc tests at $p < 0.05$).

Fresh root mass was significantly lower in the control seeds (50.41 ± 10.22 mg) than in all treatments, which clustered uniformly between 71.21 and 81.97 mg (KW: $H = 48.528$, $p < 0.001$), suggesting that even the lowest concentration of extract (0.5%) was sufficient to saturate the root fresh biomass response. Dry root mass peaked at 5% (8.54 ± 1.80 mg) and was significantly higher than the control (5.67 ± 1.29 mg) and 20% (6.79 ± 1.03 mg). For fresh and dry root mass, the BC.4 hormesis parameters were not statistically significant ($p = 0.226$ and $p = 0.660$, respectively), consistent with the plateau-like stimulatory profile observed across treated concentrations (Fig 3C and 3D).

Shoot length showed the most pronounced hormetic response among all morpho-physiological variables (KW: $H = 189.007$, $p < 0.001$): length increased from 11.30 ± 1.34 cm in the control to a maximum of 13.61 ± 1.56 cm at 1% v/v, remained statistically equivalent through 5%, but declined sharply at 20% to 9.49 ± 3.02 cm, the lowest value recorded, significantly below all other treatments (Fig. 3E). The BC.4 model fitted this response with $R^2 = 0.953$. Root length (Fig. 3F) showed a pattern paralleling shoot length: minimal at the control (6.02 ± 1.80 cm), maximal between 1 and 10% (~9.25–9.47 cm), and intermediate at 20% (7.38 ± 2.09 cm; KW: $H = 135.689$, $p < 0.001$). The BC.4 model for root length yielded $R^2 = 0.930$ and an e-value of 6.524 ± 1.665% ($p = 0.030$), although the hormesis parameter did not reach statistical significance ($f = 2.097 \pm 0.723$, $p = 0.063$); Table 2).

The root:shoot mass ratio was significantly lower in the control (0.46 ± 0.09) than in all primed treatments (0.56–0.62); KW: $H = 27.264$, $p < 0.001$; Fig. 3G), indicating that root elongation was disproportionately stimulated relative to shoot elongation by the extract, particularly at low concentrations. The seedling vigor index (SVI), which integrates both germination and seedling length, captured the full hormetic profile most clearly (KW: $H = 230.807$, $p < 0.001$; Fig. 3H). SVI was equivalent at control and 0.5% v/v (10.79 and 11.14), peaked at 1–5% (~12.59–12.74), returned to control-level values at 10% (10.82), and reached its minimum at 20% (7.95 ± 2.62). The BC.4 model for SVI yielded $R^2 = 0.976$, with a non-significant f parameter ($p = 0.370$).

Table 2 – Brain-Cousens four-parameter (BC.4) dose-response model parameters for fresh shoot mass (FSM, mg), dry shoot mass (DSM, mg), fresh root mass (FRM, mg), dry root mass (DRM, mg), root:shoot ratio (R:S), shoot length (SL, cm), root length (RL, cm), and seedling vigor index (SVI) of barley seedlings in response to seed priming with *Kappaphycus alvarezii* biostimulant (0, 0.5, 1, 2, 5, 10, and 20% v/v).

Variables	BC.4				
	b – Slope	d – Upper asymptote	e – ED50 reference	f – Hormesis magnitude	R^2
FSM	1.394 ± 0.016 (<0.001)	110.519 ± 0.633 (<0.001)	5.971 ± 0.296 (<0.001)	31.082 ± 1.705 (<0.001)	0.999
DSM	1.120 ± 0.029 (<0.001)	50.625 ± 1.813 (<0.001)	1.004 ± 0.296 (0.043)	104.276 ± 27.198 (0.031)	0.904
FRM	1.322 ± 0.124 (0.002)	11.641 ± 0.457 (<0.001)	5.969 ± 3.319 (0.170)	2.415 ± 1.590 (0.226)	0.986
DRM	1.078 ± 0.112 (0.002)	5.734 ± 0.751 (0.005)	0.611 ± 1.343 (0.680)	15.159 ± 31.164 (0.660)	0.667
R:S	1.421 ± 0.122 (0.001)	11.422 ± 0.442 (<0.001)	5.826 ± 2.414 (0.095)	2.705 ± 1.358 (0.140)	0.775
SL	1.194 ± 0.078 (<0.001)	6.081 ± 0.497 (0.001)	1.454 ± 0.827 (0.177)	8.961 ± 4.702 (0.153)	0.953
RL	1.540 ± 0.102 (<0.001)	10.703 ± 0.324 (<0.001)	6.524 ± 1.665 (0.030)	2.097 ± 0.723 (0.063)	0.930
SVI	1.012 ± 0.028 (<0.001)	0.466 ± 0.036 (<0.001)	0.251 ± 0.250 (0.390)	2.474 ± 2.350 (0.370)	0.976

Parameters: *b* = slope at inflection point; *d* = upper asymptote (response at maximal inhibitory concentration); *e* = ED_{50} (reference concentration dose); *f* = hormesis magnitude (suprabasal stimulation above upper asymptote; f > 0 indicates confirmed hormesis). Values presented as Estimate ± SE with p-value in parentheses. R^2 = coefficient of determination for model goodness of fit. Statistically significant parameters: $p < 0.05$).

3.3. Heatmap overview of morphophysiological variables

The Z-score heatmap of morpho-physiological variables (Fig. 3) provided a standardized overview of treatment effects relative to the overall distribution of each variable, enabling a direct visual comparison of response magnitudes across concentrations. A clearly biphasic pattern emerged along the concentration gradient. The control (0%) was characterized by predominantly negative Z-scores across nearly all variables, most markedly for fresh root mass (Z = −2.09), root:shoot ratio (Z = −2.09), root length (Z = −1.85), and dry root mass (Z = −1.76), indicating that untreated seedlings were consistently below the mean for all growth-related traits. In contrast, intermediate concentrations (1–5% v/v) displayed a warm-toned (positive Z-score) profile across the majority of variables, with fresh shoot mass (Z = 1.40, at 5%), dry root mass (Z = 1.45, at 5%), and root length (Z = 0.78, at 5%) reaching their highest values at this concentration range. The 20% v/v treatment produced the most heterogeneous pattern: it achieved the highest Z-score for root:shoot ratio (Z = 1.01), reflecting a disproportionate inhibition of shoot elongation relative to root elongation, while simultaneously recording the most strongly negative values for shoot length (Z = −1.87), dry shoot mass (Z = −1.71), and SVI (Z = −1.94). The 0.5% v/v treatment occupied an intermediate position, with warm tones emerging for dry shoot mass (Z = 0.57), root:shoot ratio (Z = 0.36),

and dry root mass (Z = 0.50), but remaining near-neutral or slightly negative for shoot and root lengths, suggesting that this concentration was below the threshold for stimulating elongation. Collectively, the heatmap revealed that priming effects on shoot and root length and vigor were maximized between 1 and 5% v/v, with clear evidence of inhibition beyond 10% concentration.

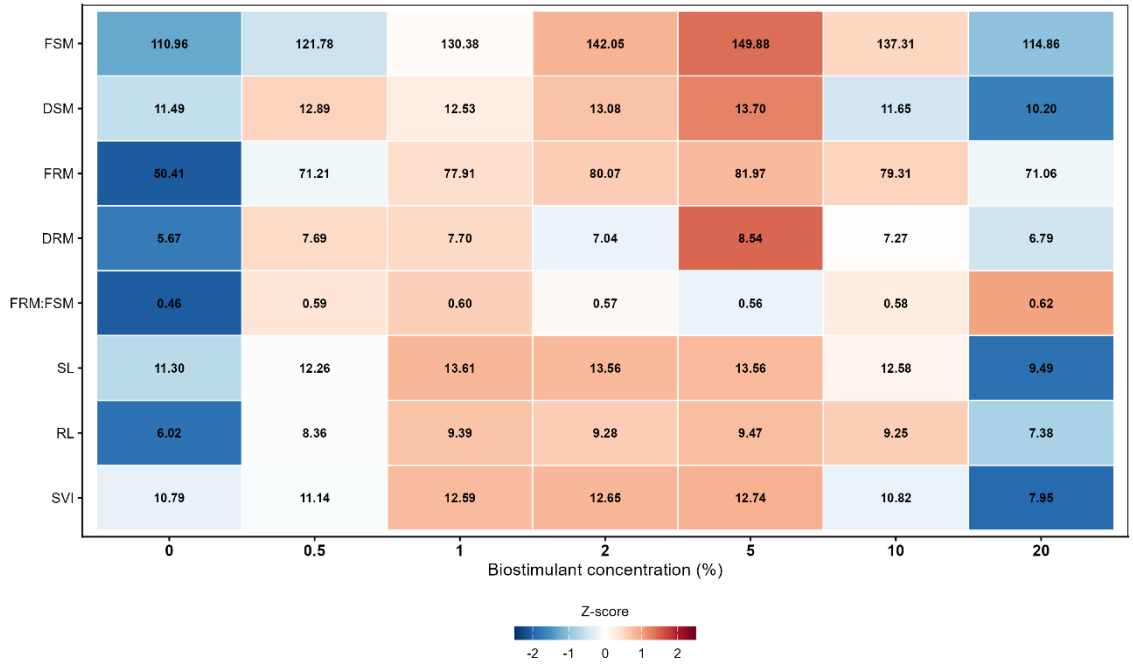

Fig. 3. Z-score heatmap of morphophysiological variables of barley seedlings in response to seed priming with *Kappaphycus alvarezii* biostimulant at seven concentrations (0, 0.5, 1, 2, 5, 10, and 20% v/v).

Fresh shoot mass (FSM, mg), dry shoot mass (DSM, mg), fresh root mass (FRM, mg), dry root mass (DRM, mg), root:shoot ratio (R:S), shoot length (SL, cm), root length (RL, cm), and seedling vigor index (SVI). Numerical values within each cell represent the mean for the corresponding variable and concentration. Color scale indicates the Z-score relative to the grand mean across concentrations: red (warm) tones denote values above the cross-concentration mean (Z > 0), and blue (cool) tones denote values below the mean (Z < 0). Variables: fresh shoot mass (mg), dry shoot mass (mg), fresh root mass (mg), dry root mass (mg), root:shoot ratio (dimensionless, fresh mass basis), shoot length (cm), root length (cm), seedling vigor index (SVI).

3.4. Photosynthetic pigments

All photosynthetic pigments studied were significantly affected by priming concentration (Chl *a*, F = 17.082; Chl *b*, F = 18.613; TChl, F = 17.590; all $p < 0.001$; KW: TCN, H = 15.134, p = 0.019). Chl *a*, Chl *b*, and TChl exhibited a consistent, non-monotonic response: values were minimal in the control seedlings (0.63 ± 0.02, 0.22 ± 0.01, and 0.85 ± 0.02 mg g^{-1}, respectively), increased with extract concentration, and peaked markedly at 2%-

treated seedlings (0.76 ± 0.03, 0.26 ± 0.01, and 1.02 ± 0.04 mg g^{-1}, respectively), before returning to intermediate values at 5–20% treatments (Fig. 4). The peak TChl was observed in plants treated with the biostimulant at 2%, representing a ~20% increase over the control. TCN followed a similar trend, with the control seedlings (0.12 ± 0.00 mg g^{-1}) being the only group significantly lower than 2%-treated seedlings (0.14 ± 0.01 mg g^{-1}; Fig. 4D). Despite these significant treatment effects, the BC.4 model parameters for all pigment variables were non-significant for the hormesis term f ($p \geq 0.387$), and R^2 values were moderate to low (0.351–0.732; Table 3), indicating that the pigment responses do not conform to a strict hormetic dose-response curve and are better described as a concentration-optimum relationship centered around 2% concentration of the biostimulant.

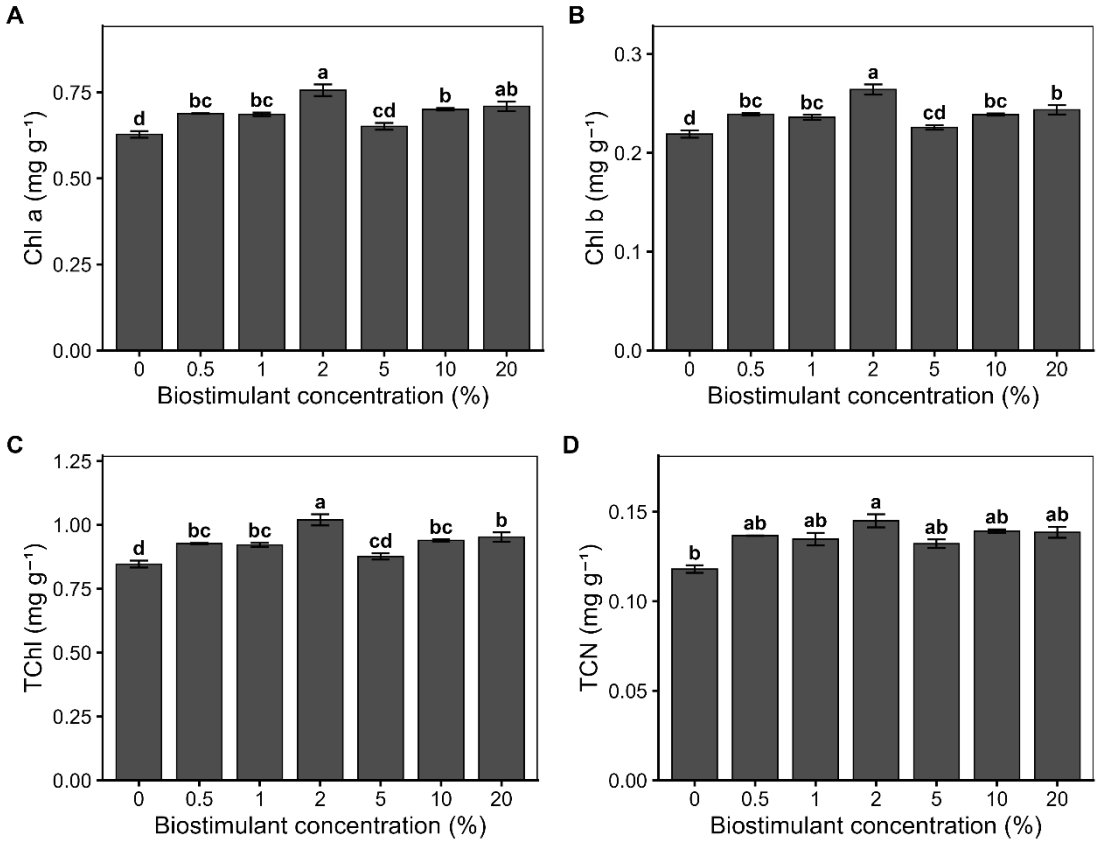

Fig. 4. Chlorophyll *a* (Chl a; A), chlorophyll *b* (Chl a; B), total chlorophyll (TChl; C), and total carotenoids (TCN; D) in shoots of barley seedlings following seed priming with *Kappaphycus alvarezii* biostimulant at seven concentrations (0, 0.5, 1, 2, 5, 10, and 20% v/v).

Bars represent means ± SE (n = 3 independent extractions). Different letters indicate significant differences (Tukey HSD or Dunn-Bonferroni, $p < 0.05$).

3.5. Primary metabolites

Total soluble sugar content (TSS) ranged from 37.36 ± 1.90 mg g^{-1} (control) to 57.27 ± 12.19 mg g^{-1} (1%), with high intra-group variability at intermediate concentrations (Fig. 5). Despite a significant overall Kruskal-Wallis result for TSS ($H = 14.715$, $p = 0.023$), post-hoc comparisons indicated no pairwise differences. Consistent with this, the BC.4 model for TSS produced a non-significant slope (b: $p = 0.707$) and very low explanatory power ($R^2 = 0.084$; Table 3), indicating the absence of a structured dose-response relationship. Total starch (TS) displayed a striking monotonic decrease with increasing biostimulant concentration ($F = 33.187$, $p < 0.001$): from 10.25 ± 0.65 mg g^{-1} in the control seedlings to a minimum of 4.21 ± 1.06 mg g^{-1} at 10%-treated seedlings, representing a 58.9% reduction (Fig. 5B). The BC.4 model described this response well ($R^2 = 0.779$; Table 3), though the hormesis parameter (f) was non-significant ($p = 0.976$), confirming a monotonically inhibitory rather than hormetic profile.

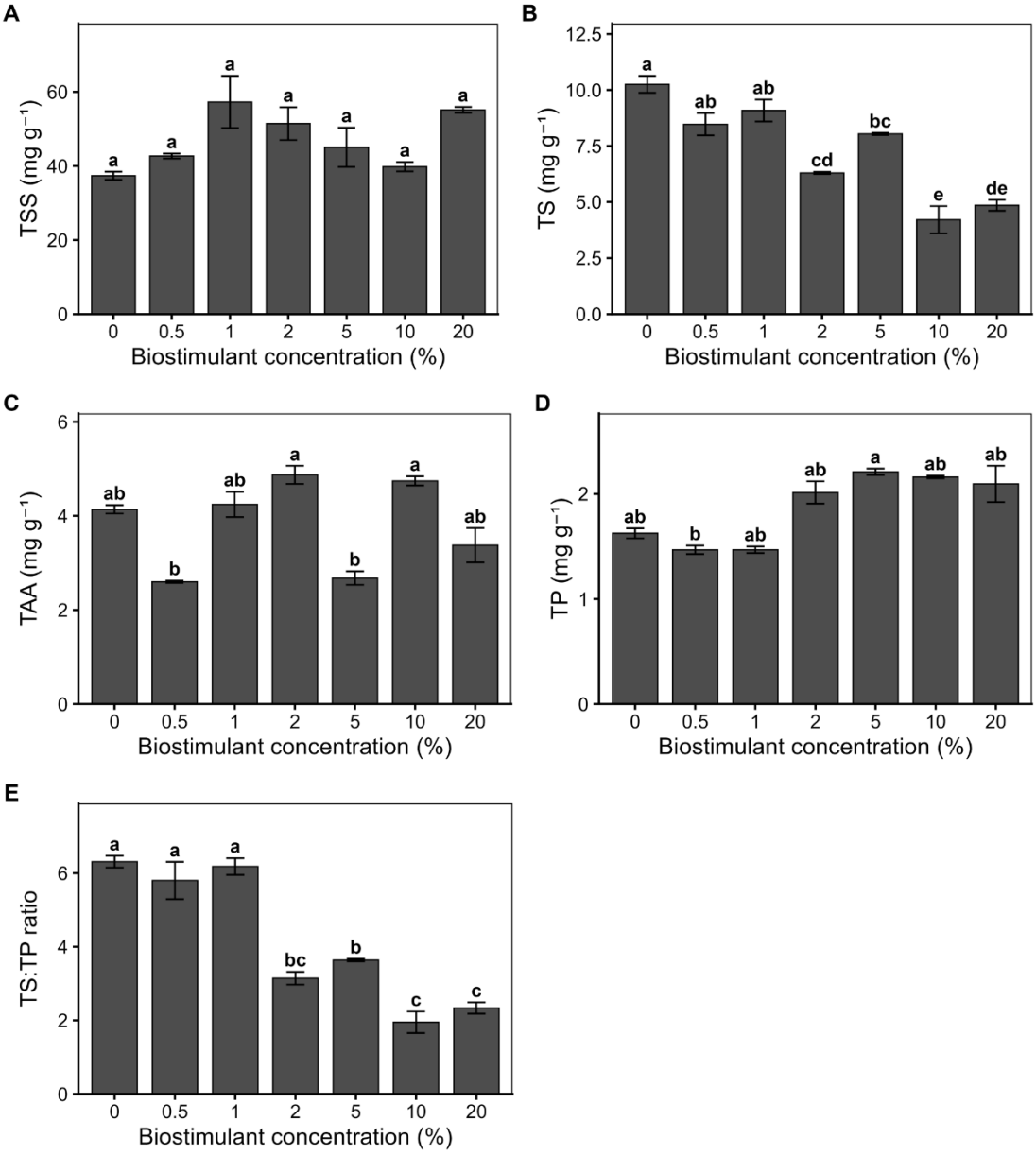

Fig. 5. Total soluble sugars (TSS; A), total starch (TS; B), total amino acids (TAA; C), total protein (TP; D), and starch:protein ratio (TS:TP; E) of barley seedling shoots following seed priming with *Kappaphycus alvarezii* biostimulant at seven concentrations (0, 0.5, 1, 2, 5, 10, and 20% v/v).

Bars represent average ± SE. Different letters denote significant differences among concentrations (Tukey HSD for TS and TS:TP ratio; Dunn-Bonferroni for the others; $p < 0.05$).

Total amino acid (TAA) also showed a significant overall effect (KW: H = 17.189, p = 0.009), with peak values at 2%- and 10%-treated seedlings (4.87 ± 0.33 and 4.74 ± 0.17 mg 100 g^{-1}) and significantly lower values in the treatments 0.5% and 5% (2.60 and 2.68 mg 100 g^{-1}; Fig. 5C). The BC.4 model for TAA did not converge to a biologically meaningful fit (R^2 =

0.091; Table 3), suggesting an irregular, concentration-dependent pattern inconsistent with a unimodal dose-response.

Table 3 – Brain-Cousens four-parameter (BC.4) dose-response model parameters for chlorophyll *a* (Chl *a*, mg g^{-1}), chlorophyll *b* (Chl *b*, mg g^{-1}), total chlorophyll (TChl, mg g^{-1}), total carotenoid (TCN, mg g^{-1}), total soluble sugars (TSS, mg g^{-1}), total amino acids (TAA, mg g^{-1}), total protein (TP, mg g^{-1}), total starch (TS, mg g^{-1}), and starch:protein ratio (TS:TP) of barley seedlings in response to the seed priming with the *Kappaphycus alvarezii* biostimulant (0, 0.5, 1, 2, 5, 10, and 20% v/v).

Variables	Brain-Cousens BC.4 parameters				
	b – Slope	d – Upper asymptote	e – ED50 reference	f – Hormesis magnitude	R^2
Chl a	1.022 ± 0.046 (<0.001)	0.630 ± 0.044 (<0.001)	0.593 ± 1.147 (0.641)	1.263 ± 2.364 (0.630)	0.420
Chl b	1.031 ± 0.060 (<0.001)	0.220 ± 0.017 (<0.001)	0.807 ± 1.894 (0.699)	0.325 ± 0.743 (0.691)	0.351
TChl	1.028 ± 0.049 (<0.001)	0.852 ± 0.061 (<0.001)	0.770 ± 1.358 (0.611)	1.321 ± 2.262 (0.600)	0.394
TCN	1.033 ± 0.035 (<0.001)	0.119 ± 0.006 (<0.001)	0.736 ± 0.762 (0.405)	0.206 ± 0.205 (0.387)	0.732
TSS	0.887 ± 2.148 (0.707)	45.297 ± 5.608 (0.004)	378.829 ± 6181.047 (0.955)	0.497 ± 2.935 (0.876)	0.084
TAA	12.260 ± 136.719 (0.934)	3.696 ± 0.686 (0.013)	21.360 ± 16.091 (0.276)	0.059 ± 0.147 (0.714)	0.091
TP	1.415 ± 0.493 (0.064)	1.459 ± 0.157 (0.003)	10.485 ± 9.654 (0.357)	0.288 ± 0.274 (0.370)	0.811
TS	0.580 ± 0.771 (0.507)	10.215 ± 1.508 (0.007)	11.592 ± 36.364 (0.771)	0.025 ± 0.777 (0.976)	0.779
TS:TP	1.291 ± 0.191 (0.007)	6.345 ± 0.983 (0.008)	0.721 ± 1.634 (0.689)	6.866 ± 23.717 (0.791)	0.864

Parameters: *b* = slope at inflection point; *d* = upper asymptote (response at maximal inhibitory concentration); *e* = ED_{50} (reference concentration dose); *f* = hormesis magnitude (suprabasal stimulation above upper asymptote; f > 0 indicates confirmed hormesis). Values presented as estimate ± SE with p-value in parentheses. R^2 = coefficient of determination for model goodness of fit. Statistically significant parameters: $p < 0.05$.

In turn, the total protein (TP) content was significantly affected by treatment (KW: H = 16.984, p = 0.009) and followed a non-monotonic pattern: lowest at 0.5 and 1% (1.47 ± 0.07 and 1.47 ± 0.05 mg g^{-1}, respectively), then increasing to a maximum at 5%-treatment (2.21 ± 0.05 mg g^{-1}), and remaining elevated in seedlings treated with higher concentrations of the biostimulant: 10 and 20% (~2.09–2.16 mg g^{-1}; Fig. 5D). The BC.4 model fitted protein content with R^2 = 0.811, though the hormesis parameter was not significant (f: p = 0.370) and the e-value was estimated at 10.5% v/v, suggesting a delayed inhibitory threshold rather than a stimulatory-then-inhibitory profile. The starch:protein ratio (TS:TP), declined sharply and significantly from 6.31 ± 0.28 in the control to 1.95 ± 0.50 at 10%-treated seedlings (ANOVA:

F = 51.314, p < 0.001; BC.4: R^2 = 0.864; Table 3), indicating a progressive shift in carbon/nitrogen partitioning with increasing extract concentration.

3.6. Z-score heatmap of biochemical variables

The Z-score heatmap of biochemical variables (Fig. 6) revealed distinct metabolic signatures for each concentration, with pigment and primary metabolite variables exhibiting contrasting dose-dependent trajectories. Photosynthetic pigments (Chl *a*, Chl *b*, TChl, and TCN) showed a coherent pattern: the control exhibited the most negative Z-scores across all four pigment variables (ranging from Z = −1.33 for Chl *b* to Z = −2.00 for TCN), while the 2% treatment was the sole concentration yielding strongly positive Z-scores for all four pigments simultaneously (Z = +1.64, +1.82, +1.69, +1.19 for Chl *a*, *b*, TChl, and TCN, respectively), clearly distinguishing it as a pigment-stimulatory optimum. Higher concentrations of the biostimulant (i.e., 5 to 20%, v/v) returned to intermediate or near-zero pigment scores, confirming a narrow optimal concentration window for photosynthetic pigment enhancement.

For primary metabolism, an opposing pattern was observed between TS and TP. Z-scores for TS were highest at 0% (Z = +1.31) and 0.5% (Z = +0.51) in the seedlings and decreased with increasing biostimulant concentration, reaching the most negative values at 10% (Z = −1.38) and 20% (Z = −1.09). This mirror image was captured by the starch:protein ratio, which was strongly positive at the 0% treatment (Z = +1.13) and progressively negative at 10% and 20% treatments (Z = −1.20 and −1.00, respectively). TP, by contrast, was most negative in seedlings treated with the biostimulant at 0.5 and 1% (Z ≈ −1.19) and most positive in higher concentrations of that bioproduct, e.g., 5 and 10% (Z = +1.05 and +0.90). Together, these opposing trajectories describe a progressive shift in the carbon-to-nitrogen metabolic allocation with increasing biostimulant concentration: seedlings primed with low concentrations retained a starch-dominant metabolism resembling the control, whereas those primed with intermediate-to-high concentrations of the biostimulant exhibited an enhanced protein-accumulating phenotype at the expense of starch reserves. TSS and TAA presented more variable Z-score profiles without a consistent directional trend across concentrations, consistent with the absence of structured dose-response fits observed in the BC.4 modelling.

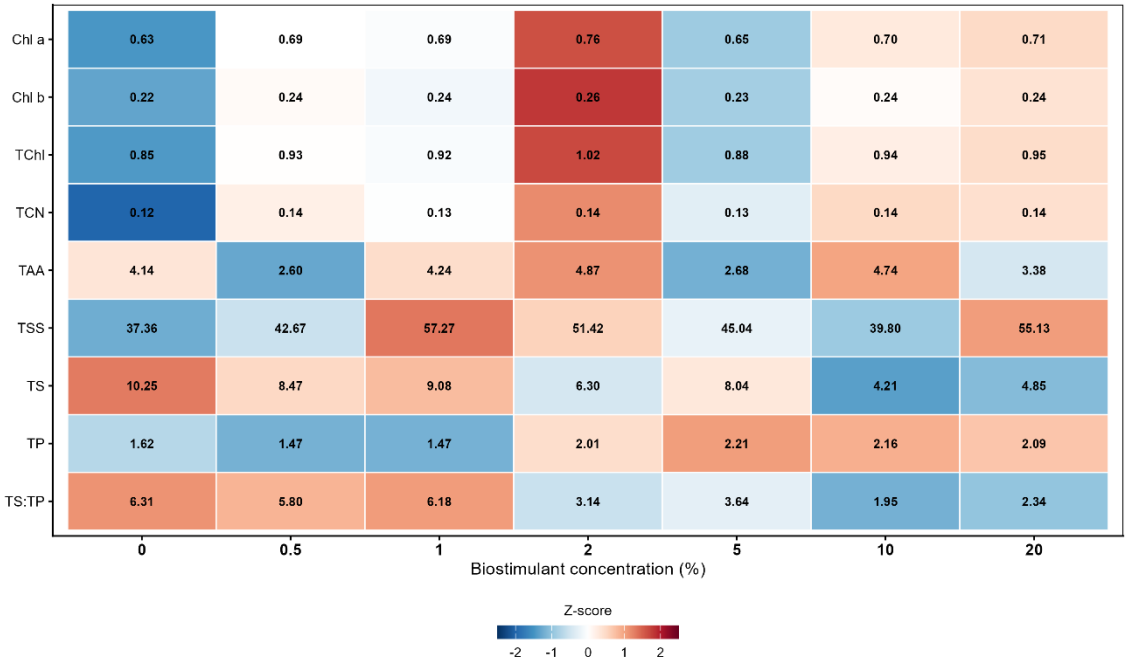

Fig. 6. Z-score heatmap of biochemical variables of barley seedling shoots in response to seed priming with *Kappaphycus alvarezii* biostimulant at seven concentrations (0, 0.5, 1, 2, 5, 10, and 20% v/v).

Numerical values within each cell represent the treatment mean in original units. Color scale represents the Z-score relative to the cross-concentration grand mean for each variable (each row is standardized independently): red (warm) tones indicate values above the row mean ($Z > 0$); blue (cool) tones indicate values below ($Z < 0$). Variables (rows): chlorophyll *a* (Chl *a*, mg g^{-1}), chlorophyll *b* (Chl *b*, mg g^{-1}), total chlorophyll (TChl, mg g^{-1}), total carotenoid (TCN, mg g^{-1}), total soluble sugars (TSS, mg g^{-1}), total amino acids (TAA, mg g^{-1}), total protein (TP, mg g^{-1}), total starch (TS, mg g^{-1}), and starch:protein ratio (TS:TP).

3.7.Principal component analysis

PCA of the morphophysiological variables (Fig. 7) explained 92.5% of total variance of the data set across the first two principal components (PC1: 68.4%; PC2: 24.1%). PC1 was broadly representative of seedling growth performance, with near-equal contributions from RL (18.43%), FSM (18.18%), SL (16.44%), DRM (15.35%), FRM (15.06%), and DSM (15.00%). PC2 was largely determined by germination percentage (53.95%) and, to a lesser extent, FRM (15.31%), indicating that variance in germination, although overall limited, was orthogonal to the main axis of seedling growth. Treatment scores along PC1 separated the concentrations in accordance with their growth-stimulatory potential: the control (0%) occupied PC1- and PC2+ and the 20% treatment PC1- and PC2-, consistent with their below-mean and near-mean growth scores across most variables, respectively, whereas aqueous concentrations between 1 and 5%

v/v projected toward the PC1+ and PC2+, reflecting their consistently superior shoot and root elongation, biomass accumulation, and seedling vigor. The 0.5% treatment occupied an intermediate PC1+, slightly below the 1–5% cluster. The 20% treatment was notably separated from the remaining concentrations, driven by its lower germination percentage (i.e., 84%) relative to the cluster formed by 0–5% (91%–95%), a difference not captured by post-hoc pairwise comparisons but numerically consistent.

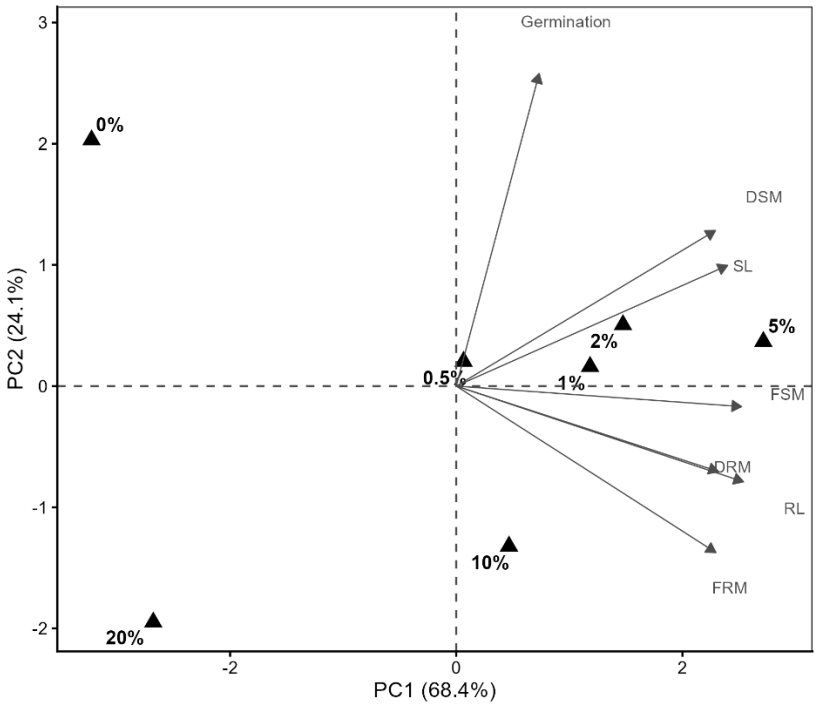

Fig. 7. Principal component analysis (PC1 vs PC2) of morpho-physiological variables of barley (seedlings in response to seed priming with *Kappaphycus alvarezii* biostimulant (0, 0.5, 1, 2, 5, 10, and 20% v/v).

Data were Z-score standardized (treatment means, n = 20). FSM = Fresh shoot mass (mg), DSM = dry shoot mass (mg), FRM = fresh root mass (mg), DRM = dry root mass (mg), R:S = root:shoot ratio, SL = shoot length (cm), RL = root length (cm), and germination (%).

PCA of the biochemical variables investigated (Fig. 8) explained 78.6% of total variance of the data set, with the first two principal components (PC1: 61.6%; PC2: 17.0%). Considering the contributions of a third component (PC3: 12.0%, Fig.S2, supplementary material) to the descriptive model, the cumulative explained variance reached 90.5%. PC1 was dominated by photosynthetic pigments, with Chl *a* (19.53%), TChl (19.30%), Chl *b* (18.32%), and TCN (17.97%) collectively accounting for 75.1% of PC1's loadings. TS contributed modestly to PC1 (11.82%), while TP (3.47%) and TAA (3.50%) had minimal effect on the

total variance of the dataset. In turn, PC2 was governed by TP (51.52%), TS (23.37%), and TSS (18.88%), collectively representing primary carbon and nitrogen metabolites, and thus interpreted as a metabolic partitioning axis.

The ordination of treatments along PC1 was primarily driven by their pigment contents: the 2%-treated seedlings projected toward PC1+ and PC2+ pole, consistent with its peak Z-scores for all four pigments, while the control seedlings (0%) occupied the most negative PC1-position, reflecting its minimum pigment content. Additionally, the 5% treatment was displaced in PC1- PC2- direction, consistent with its below-average pigment Z-scores. 0.5%- and 1%-treated seedlings were distinctly separated from other concentrations by their high TS content and low TP content, occupying PC1- PC2+ pole, while 10%- and 20%-treated were projected toward PC1+PC2- pole, due to their high TP content.

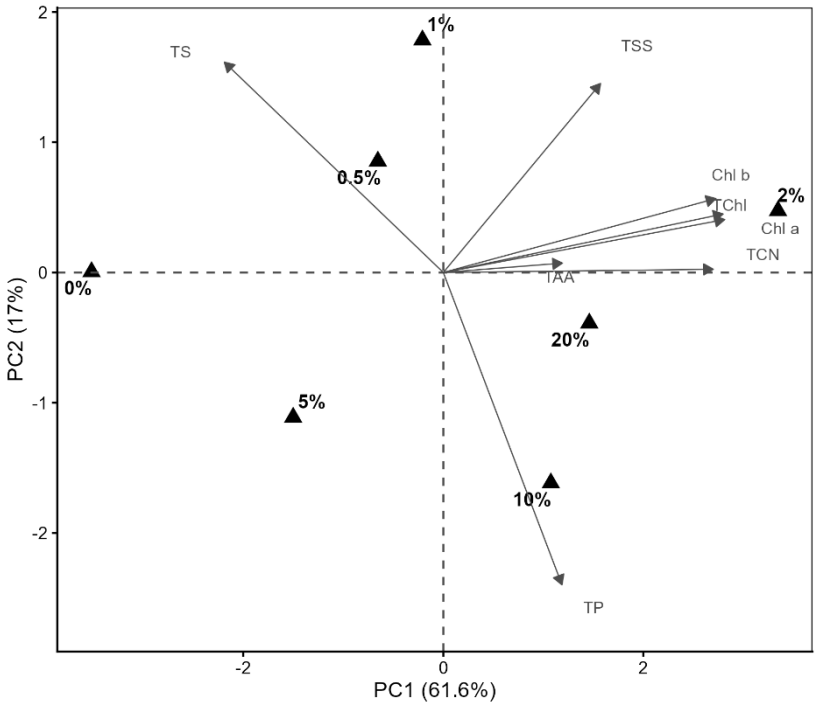

Fig. 8. Principal component analysis (PC1 vs PC2) of biochemical variables of barley seedlings in response to seed priming with *Kappaphycus alvarezii* biostimulant (0, 0.5, 1, 2, 5, 10, and 20% v/v).

Data were Z-score standardized (treatment means). Variables: Chl a = chlorophyll a (mg g^{-1}); Chl b = chlorophyll b (mg g^{-1}); TChl = total chlorophyll (mg g^{-1}); TCN = carotenoids (mg g^{-1}); TSS = total soluble sugars (mg g^{-1}); TS = total starch (mg g^{-1}); TP = total protein (mg g^{-1}); TAA = total amino acids (mg g^{-1}).

4. Discussion

4.1. *K. alvarezii* extract as biostimulant

The chemical profile of the *K. alvarezii* biostimulant (Table 1) provides the interpretive anchor for the entire dose–response landscape observed in this study. With total nitrogen below the detection limit, the extract cannot function as a conventional nitrogenous fertilizer. Instead, its effects on barley seedling physiology must be attributed to the complex mixture of signaling molecules and osmotically active solutes it delivers. Chief among these are kappa-carrageenan oligosaccharides, which are released during the mechanical processing of *K. alvarezii* biomass and have repeatedly been shown to elicit growth-promoting and defense-priming responses in plants at nanomolar to micromolar concentrations [11,28]. The extract also contained appreciable quantities of total phenolic compounds (16.78 mg 100 g^{-1}) and amino acids (3.23 mg 100 g^{-1}), both of which have been implicated in the biostimulant activity of seaweed formulations, acting as antioxidants, metal chelators, and precursors of secondary messengers [3,29].

The macronutrient profile reinforces this interpretation. Potassium, at 1.62% dry weight, is a well-documented contributor to the growth-promoting properties of *K. alvarezii* sap [5]. In the present seed-priming context, however, the absolute quantity of K^+ delivered to an individual seed was modest (~0.003–0.125 mg mL^{-1} across the 0.5–20% range, Table 1), and the dominant inorganic solute was sodium, not potassium. Sodium concentrations in the priming solutions ranged from 0.012 mg mL^{-1} (0.5 %) to 0.494 mg mL^{-1} (20%). At low concentrations, this modest Na^+ load may serve as a eustress signal, activating ion-transport systems and osmotic adjustment pathways that prime the seedling for more robust post-germinative growth [30]. At high concentrations, however, the same ion becomes an osmotic constraint, a transition that underlies the biphasic dose–response documented below. This dual role of Na^+, eustressor at low ionic loads and inhibitor at high concentrations, is well-documented for halopriming in small-grain cereals: Ben Youssef et al. [31] demonstrated that priming barley seeds with 50–100 mM NaCl enhanced germination uniformity and early seedling growth relative to unprimed controls, while concentrations above 150 mM progressively inhibited radicle elongation, prefiguring the concentration-response pattern observed here. Collectively, the compositional data establish that the *K. alvarezii* extract operates as a genuine biostimulant, in which growth modulation is driven by bioactive signaling rather than by direct nutritional supplementation.

4.2. Hormetic seedling responses to *K. alvarezii* biostimulant

Application of the Brain-Cousens four-parameter hormesis model to the morphophysiological dataset yielded statistically robust evidence of a biphasic, concentration-dependent response. Fresh shoot mass provided the most unambiguous hormetic signature (Table 2): the BC.4 model explained 99.9 % of the variance, and the hormesis parameter *f* was highly significant, confirming a low-dose stimulation substantially above the upper asymptote. The inflection-point dose placed the boundary between stimulation and incipient inhibition near the 6 % mark, a finding corroborated by the SVI, which peaked at 1–5 % and declined at 10 % and above. This 2–5 % stimulatory window aligns remarkably well with the optima reported for seaweed-extract priming in other crops. Dutta et al. [11] observed maximal germination improvement and seedling vigor in *Capsicum frutescens* primed with 2–5 % *K. alvarezii* biostimulant, and Radwan et al. [12] reported enhanced germination and antioxidant enzyme activity in *Citrullus lanatus* seeds primed with *Ulva lactuca* extract at comparable dilution rates. The convergence of these optima across taxonomically diverse species suggests a conserved sensitivity window for seaweed-derived elicitors in seed priming.

The inhibitory phase that emerged at 10–20 % treatments can also be mechanistically interpretable through the lens of salt-stress physiology. At 20 %, the priming solution delivered approximately 0.494 mg Na^+ mL^{-1} (≈21.5 mM Na^+), a concentration that approaches the threshold known to impose osmotic constraints during imbibition and to interfere with the reactivation of pre-germinative metabolism in glycophytic cereals [32]. Whereas lower doses may have acted as a mild eustress signal, this Na^+ load falls within a range that induces osmotic and ionic stress. Consistent with this, shoot length, dry shoot mass, and seedling vigor declined progressively at the highest concentration, while germination remained relatively unaffected, a pattern characteristic of salt-induced inhibition of post-germinative growth rather than of radicle emergence. This differential sensitivity mirrors observations by El-Katony et al. [33], who reported that durum wheat landraces maintained high germination at −0.750 MPa yet exhibited contrasting early-growth responses to salt versus osmotic stress, confirming that germination can be relatively preserved while seedling development undergoes marked alterations. The overall pattern, stimulation at low doses transitioning to inhibition at higher ones, is the defining signature of hormesis and underscores the importance of rigorous dose–response modelling in biostimulant research, where testing too narrow a concentration range often obscures the biphasic relationship [14].

Germination percentage, although significantly affected by biostimulant treatments, did not separate into distinct groups by post hoc pairwise testing, with all concentrations remaining between 84–95%. This pattern is consistent with the well-established relative tolerance of seed germination *stricto sensu* to moderate osmotic stress: seed coat permeability and embryo viability are maintained under conditions that nonetheless impair subsequent elongation growth [33,34]. The mild numerical differences in the 10%- and 20%-treated seeds (86% and 84%, respectively, versus 95% in the control) may represent a biologically meaningful effect that the experimental design was insufficiently powered to resolve statistically, due to limited replication and high intra-group variability. Notably, the SVI captured this threshold because it integrates elongation and germination and was the variable most sensitive to the inhibitory phase.

The preferential stimulation of root growth relative to shoot growth further reinforces the hormetic interpretation. Fresh root mass reached its statistical ceiling at 0.5% concentration, whereas shoot mass continued to increase up to 5%, resulting in a root:shoot ratio consistently and significantly higher in all extract-primed treatments (0.56–0.62) than in the control (0.46). The root-growth-promoting activity of seaweed extracts is mediated by auxin-like oligosaccharides and polyphenols that activate lateral root initiation at low concentrations, but lose or reverse their activity at higher ones [2,34]. Notably, root mass saturation occurred at a lower concentration threshold than shoot mass, a pattern consistent with the higher sensitivity of root meristems to auxin-like signals during imbibition and with the physical proximity of the radicle to the priming solution during the 12h contact period. Importantly, the elevated root:shoot ratio was maintained even at 20%-treated seedlings, where shoot elongation was markedly inhibited, indicating that roots retain a growth advantage under the ionic conditions imposed by the highest extract concentrations, consistent with the greater capacity of root cells for osmotic adjustment in Poaceae [35].

The seedling vigor index (SVI) captured the dose–response landscape in its most integrated form, combining germination and elongation into a single metric that simultaneously reflects the stimulatory and inhibitory limbs of the hormetic curve. SVI was statistically equivalent between the 0% and 0.5% treatments, peaked at 1–5%, returned to control-level values at the 10% treatment, and reached its minimum at the 20% treatment, a hormetic trajectory that no single morphological variable captured as completely. Ben Youssef et al. [31] demonstrated that priming with KNO_3 and $CaCl_2$ significantly enhanced seedling length and dry biomass in barley under salt stress, with inorganic treatments clearly outperforming

unprimed controls, while concentrations above the tested optima progressively narrowed this advantage, a response profile mechanistically analogous to the one documented here for an organic priming matrix. Similarly, Dutta et al. [11] reported that *K. alvarezii* sap priming of bird's eye chilli seeds (*Capsicum frutescens*) produced a peaked vigor-index response at intermediate concentrations, declining at higher sap dilutions, and Makhaye et al. [36] documented a concentration-dependent SVI improvement in okra (*Abelmoschus esculentus*) bioprimed seeds with seaweed extracts. The BC.4 model's moderate fit for SVI ($r^2 = 0.976$), compared with the near-perfect fit for shoot fresh mass ($r^2 = 0.999$), reflects the additional variance explained by the germination component of the index, which was not significantly differentiated across treatments in post hoc testing. Nevertheless, the SVI data collectively confirm that the extract's net effect on seedling performance, growth integrated over germination, is unequivocally stimulatory at 1–5% and inhibitory beyond 10% concentration.

4.3. Concentration-dependent shift in carbon–nitrogen partitioning

The most striking biochemical signature of *K. alvarezii* priming of barley seeds was the progressive, concentration-dependent decline in total starch content, which fell by 59 % between the control and the 10% concentration, accompanied by a concomitant inversion of the starch:protein ratio. This monotonic shift in carbon:nitrogen partitioning constitutes a biochemical fingerprint of the metabolic reprogramming induced by seed priming, and the Z-score heatmap captures this transition (Fig. 6), with the red-toned starch-dominant profile of the control giving way to the blue-toned protein-dominant profile at 10–20% treatments. The nearly 60% depletion of starch observed here strongly suggests that *components of the K. alvarezii extract* enhanced starch catabolism during the 12-h priming window. In barley aleurone cells, starch mobilization is canonically triggered by the GA→DELLA→α-amylase signaling cascade: gibberellins (GAs) released by the embryo relieve DELLA-mediated repression, allowing α-amylase gene expression and secretion into the starchy endosperm [37]. The seaweed-derived components present in the extract may activate this cascade either directly, through GA-like activity of oligosaccharides or terpenoid fractions, or indirectly, through a GA-independent pathway. Evidence for the latter was provided by Rayorath et al. [37], who demonstrated that *A. nodosum* extract induced α-amylase activity in de-embryonated

barley half-seeds even in the complete absence of exogenous GA_3, confirming that seaweed bioactives can bypass the hormonal trigger and directly activate aleurone hydrolytic capacity.

Furthermore, the magnitude of starch depletion observed here is notably larger than that reported for conventional hydropriming or osmopriming in cereals, where residual starch typically declines by 20–35% relative to dry, unprimed seeds after equivalent imbibition periods [38,39]. This amplified starch mobilization suggests that the bioactive fraction of the *K. alvarezii* extract, particularly oligo-carrageenans and phenolic compounds, confers an additive or synergistic effect on amylolytic activity beyond what simple hydration alone achieves. A mechanistically analogous amplification was demonstrated by Lemmens et al. [38], who showed that hydro-primed wheat exhibited 2–3-fold higher α-amylase activity after 5 days of sprouting than dry seeds, with primed grains showing a significantly greater α-amylase induction already during the imbibition phase. The seaweed extract may compress this temporal progression, front-loading amylase activation within the 12-h priming window.

The non-monotonic protein response, lowest at 0.5–1% treatments, peaking at plants treated with the biostimulant at 5%, and remaining elevated at higher concentrations, is best interpreted as the temporal superimposition of two competing processes during imbibition [40]. Early in the priming window, cysteine proteases (HvPap-1, EP-A, EP-B) hydrolyze hordein storage proteins in the aleurone and scutellum, transiently reducing the extractable protein pool [41]. While biostimulant concentration increases, the extract's bioactive compounds, possible oligo-carrageenans that act as elicitors of endogenous hormone biosynthesis [28] and betaine-like molecules with cytokinin-like activity [42], progressively upregulate translational machinery and nitrogen-assimilation pathways, yielding the net protein gain observed at 5% treatment, consistent with the activation of protein biosynthesis reported in maize treated with *K. alvarezii* homogenate [43].

4.4. Chlorophyll and carotenoid enhancement

The photosynthetic pigments responded to priming along a concentration-optimum curve distinct from the morpho-physiological hormetic profile. Total chlorophyll content peaked at 2% in treated seedlings (>20% over the control), whereas higher biostimulant concentrations (i.e., 5–20%) returned chlorophyll levels to values statistically indistinguishable from the control. The BC.4 model did not return a significant hormesis parameter for any

pigment variable, confirming that pigment biosynthesis follows a unimodal rather than biphasic dose–response. This narrower stimulatory window implies that the signaling pathways governing chloroplast development and pigment accumulation are more tightly regulated, and more susceptible to interference at supra-optimal concentrations, than those driving cell expansion and biomass accrual. The observed result is most plausibly attributed to the betaine fraction of the seaweed extract that delays senescence-associated chloroplast catabolism and upregulates genes encoding enzymes in the tetrapyrrole biosynthetic pathway [42]. Additionally, oligo-carrageenans derived from kappa-carrageenan have been reported to upregulate light-harvesting complex proteins and photosynthetic carbon assimilation genes in *Arabidopsis thaliana* [44], providing an oligosaccharide-mediated route to chlorophyll accumulation independent of betaine activity. The decline in pigment content noted in treatments at concentration 5% or higher is consistent with ionic interference with chloroplast biogenesis at elevated Na^+ loads, as sodium in excess is known to compete with magnesium (the central atom of the chlorophyll molecule) for membrane binding sites, and to disrupt thylakoid membrane organization in glycophytic species [45].

Collectively, the biochemical data suggest that *K. alvarezii* priming reprograms the metabolic trajectory of the developing seedling, establishing a biochemical state that mirrors the profile of seeds with intrinsically high vigor. This concept of 'metabolic priming memory', in which the biochemical state established during imbibition persists and shapes post-germinative development, is increasingly recognized as the central mechanism linking pre-sowing treatments to downstream agronomic gains in vigor, stand uniformity, and stress tolerance [34]. The present data provide, to our knowledge, the first documentation of this metabolic signature in barley seeds treated with a red macroalgal extract, and lay the groundwork for mechanistic studies using enzyme activity assays and proteomics to dissect the specific pathways involved.

4.5. Limitations, agronomic implications, and future perspectives

The present study was conducted under controlled laboratory conditions, which, while enabling rigorous dose-response characterization, do not replicate the edaphic variability, temperature fluctuations, and biotic pressures of field environments. Validation trials under agronomic conditions are therefore necessary before operational recommendations can be

prescribed. The biochemical analyses relied on bulk metabolite quantification, and the mechanistic model proposed here, centered on GA-driven starch mobilization and competing proteolytic versus anabolic protein dynamics, awaits confirmation through targeted enzyme activity assays (α-amylase, cysteine proteases) and phytohormone profiling. Additionally, the biogeographical and seasonal variability in *K. alvarezii* biochemical composition documented for the Santa Catarina cultivation system [47,48] suggests that the optimal concentration window identified here (2–5%) should be validated across different harvest seasons before being incorporated into standardized protocols.

Notwithstanding these limitations, the convergence of morpho-physiological and biochemical optima at 2–5%, achievable with a locally produced, low-cost marine biomass, positions *K. alvarezii* biostimulant as an agronomically viable, ecologically benign seed-priming agent for barley production in Brazil, with particular appeal in the context of escalating synthetic input costs and expanding regulatory frameworks for biostimulant use in cereal cropping systems.

5. Conclusions

This study, to the best of our knowledge, provides the first dose-response characterization of the *K. alvarezii* biostimulant as a seed-priming agent for barley, employing the Brain-Cousens four-parameter hormesis model across seven concentrations (0-20%, v/v). A biostimulant activity window of 2% to 5% was identified, within which shoot and root biomass, seedling elongation, vigor index, and photosynthetic pigment content were maximized, while germination remained unimpaired across all concentrations. Root fresh mass production reached its maximum at 0.5% concentration, indicating that root-system enhancement is achievable with minimal extract use. A concentration-dependent metabolic reprogramming was evidenced by a 59% decline in total starch content, paralleled by an inversion of the starch: protein ratio from 6.31 to 1.95 between the control plants and the 10%-treated ones; a finding interpreted as the progressive activation of pre-germinative anabolic metabolism mediated by the bioactive fraction of the extract. At 20% concentration of biostimulant, the elevated ionic load of the priming solution imposed osmotic constraints that suppressed elongation while preserving germination, a canonical hormetic inhibitory response. Together, these findings establish a mechanistically coherent, quantitatively defined basis for the use of *K. alvarezii* biostimulant in barley seed priming.

Acknowledgements

We would like to thank VerdeMar Ltda. for kindly providing the commercial *K. alvarezii* biostimulant for this study, and Ambev S.A. (Companhia de Bebidas das Américas) for kindly supplying the barley seeds used in this study.

CRediT author statement

FSD: Conceptualization, Methodology, Investigation, Formal analysis, Data Curation, Writing – Original Draft. ERO: Investigation, Writing – Review & Editing. AN: Writing – Review & Editing. SM: Writing – Review & Editing. MM: Supervision, Writing – Review & Editing, Funding acquisition.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding sources

Research was supported by grant 88887.696139/2022-00 from the Coordination for the Improvement of Higher Education Personnel, Brazil (CAPES - Finance Code 001) to F.S.D; by grants 306495/2023-6 (M.M.) and 303956/2023-2 (E.R.O.) from the National Council for Scientific and Technological Development, Brazil (CNPq); and by grant 2023/03886-1 to A.N. from the São Paulo Research Foundation (FAPESP).

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process.

During the preparation of this work the author(s) used Claude (Anthropic) in order to assist with grammatical revision and validation of R code syntax. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Data availability

Data will be made available on request.

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Capítulo 4 – Aplicação foliar do extrato aquoso de *Kappaphycus alvarezii* em plantas de cevada (*Hordeum vulgare* L.)

Journal of Applied Phycology
https://doi.org/10.1007/s10811-026-03883-z

RESEARCH

A *Kappaphycus alvarezii* aqueous extract improves yield and kernel quality in barley (*Hordeum vulgare* L.)

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Received: 20 January 2026 / Revised: 19 April 2026 / Accepted: 25 April 2026

Abstract
The search for sustainable agricultural inputs has increased the interest in biostimulants based on macroalgae. An aqueous extract of *Kappaphycus alvarezii*, a red seaweed rich in bioactive compounds, has emerged as a promising biostimulant for various crops, in Brazil, but its effects on barley (*Hordeum vulgare* L.) remain underexplored. This study investigated the influence of foliar application of different concentrations, i.e., 0, 1, 2, 5, and 10% v/v, of a crude aqueous extract, derived from biomass cultivated in Santa Catarina, southern Brazil. The morphology, yield, and biochemical parameters of leaves and kernels, from treated plants in pots, in a greenhouse were measured. In contrast to the growth-promoting effects reported for other extracts of seaweeds, this *Kappaphycus* extract did not alter the plant architecture, but selectively enhanced reproductive output, suggesting an optimization of resource allocation towards grain development. It was seen that grain set increased at lower concentrations (1–2%) and the thousand-kernel weight at higher doses (5–10%). Biochemically, this specific extract elevated leaf amino acids by up to 47.4% at the 1% concentration, as compared to control. In addition, treatments selectively enhanced kernel qualities, including starch (+19.3%, at 5%), protein (+10.3%, at 10%), and amino acids (+56.6%, at 1–2%), while maintaining germination viability above 98.5%. PCAs revealed a clear segregation, isolating the control from all treatments, which formed distinct clusters in all parameters. The results demonstrated that the *K. alvarezii* extract used in this study could be an effective tool for enhancing barley kernel quality, particularly for the malting industry, by optimizing reserve accumulation, without compromising plant morphology and seed germination.

Keywords Seaweed aqueous extract · *Kappaphycus alvarezii* · Marine bioinputs · Rhodophyta · Sustainable agriculture · Physiological responses · Poaceae · Barley

Introduction

The search for sustainable agricultural inputs has gained global prominence, driven by the need to reduce reliance on chemical fertilizers and mitigate environmental impacts (Brunelle et al. 2024). In this context, biofertilizers and biostimulants derived from macroalgal extracts have emerged as promising alternatives (Munaro et al. 2021; Nanda et al. 2022; Sario et al. 2025). These seaweed-derived inputs are recognized for their richness in bioactive compounds (e.g., plant hormones, polysaccharides, and micronutrients) that stimulate crop plant growth and enhance resistance (Ali et al. 2021a; Shukla et al. 2025), across all plant development stages, from germination to post-harvest (Ali et al. 2021b).

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Published online: 02 June 2026

Among species of macroalgae, the rhodophyte *Kappaphycus alvarezii* (Doty) L.M.Liao stands out as the fifth most cultivated seaweed in the world (Nunes et al. 2024). This macroalga has significant industrial applications, primarily due to the presence of the polysaccharide kappa carrageenan (Rudke et al. 2020). However, it has recently also begun to be used as an agricultural input (Layek et al. 2015; Kumar et al. 2020; Vaghela et al. 2022; 2023a; 2023b), due to its content of growth-promoting compounds (Vaghela et al. 2022), defense-inducing agents against stress (Patel et al. 2018; Trivedi et al. 2018), and high levels of soluble potassium (Karthikeyan and Shanmugam 2017; Gelli et al. 2020). Consequently, it has a pronounced effect on productivity across various crops, including sugarcane, soybean, and cereals such as maize, rice, and wheat (Nunes et al. 2024). It is crucial to note, however, that the biochemical composition and efficacy of such seaweed extracts are not uniform, as biogeographical and seasonal factors inherently influence them. The biochemical profile of the *K. alvarezii* biomass, and consequently its extracts, varies with the environmental conditions of its geographic origin (Nunes et al. 2025a) and the season of cultivation (Schneider et al. 2026). A clear understanding of this variability is therefore essential for developing standardized, reliable biostimulants that meet regulatory benchmarks and perform consistently in agricultural systems.

Barley (*Hordeum vulgare* L.) is one of the oldest crops and most widely cultivated cereals globally. It is used extensively for human consumption, animal feed, and malt production (Ross et al. 2023). Despite their widespread use in agricultural research, the potential of seaweed extracts in barley studies remains underexplored. A particular area of interest lies in barley's responsiveness to different types and concentrations of seaweed extracts. For instance, applications of extracts from the brown seaweed *Ascophyllum nodosum* (L.) Le Jolis have been shown to enhance germination rates, seedling vigor, and overall plant growth in barley (Möller and Smith 1999; Rayorath et al. 2008; Goñi et al. 2021), and to influence germination, radicle and plumule growth, and seedling development (Kaya et al. 2024). In another study, the extract of *Ecklonia maxima* (Osbeck) Papenfuss improved nitrogen-use efficiency, resulting in higher yields and lower fertilizer use (Cozzolino et al. 2021). These findings underscore the significant role of seaweed extracts in enhancing barley performance, demonstrating their potential as effective tools for sustainable crop management. However, despite the extensive use of *K. alvarezii* in other cereals, its effects on barley yield, partitioning, and grain biochemical quality remain unknown.

This study aimed to evaluate the effects of a specific aqueous extract of *K. alvarezii* biomass cultivated in Santa Catarina, southern Brazil, on the yield components and kernel quality in treated barley (var. Rubi). To this end, plants were treated with different concentrations (i.e., 0, 1, 2, 5, and 10% v/v) of the aqueous extract. Agronomic parameters, including plant architecture and kernel formation, were assessed. Furthermore, comprehensive biochemical analyses were conducted on both vegetative tissues and mature kernels to determine changes in quality-related metabolites.

Material and methods

Experimental conditions

The experiment was conducted in a greenhouse at the Epagri Training Center (CETRE) in Florianópolis, Santa Catarina, Brazil, from 19 June to 23 October 2024. Seeds of barley (*Hordeum vulgare* var. Rubi) were purchased from Ambev S.A. (Companhia de Bebidas das Américas) and, when tested, showed a germination rate of 98–100%. Before sowing, the seeds were surface-disinfected with 2% sodium hypochlorite for 6 min, followed by 6 rinses with distilled water. This standardized protocol effectively eliminated surface pathogens without compromising seed vigor or germination potential. The disinfected seeds were dried overnight in an oven at 30 °C with forced-air circulation (Brasil 2018).

The seeds were sown in 10-L (30 cm diameter) pots filled with a commercial medium (TN Mix, Agrinobre, Brazil) pre-fertilized with 5 g of NPK (10:10:10) per pot. Sowing was performed by placing 8 seeds per pot, distributed in four equidistant holes. Seedlings were thinned to a final density of four plants per pot on the seventh day after emergence.

The aqueous extract of *K. alvarezii* was provided by VerdeMar® (Palhoça, Santa Catarina, southern Brazil), from biomass produced locally during the autumn. This raw liquid, obtained directly from the mechanical processing of fresh algal biomass, was used as the 100% stock solution for preparing the treatment dilutions. The biochemical characterization of the extract was performed according to the methods described by Nunes et al. (2025a). A detailed profile of its biochemical and nutrient composition is provided in Table 1.

A completely randomized design was adopted with eight replicates (pots) per treatment. The treatments consisted of foliar applications at concentrations of 1, 2, 5, and 10% (v/v), extract diluted in water, with a control (0%, water only). Based on the total solid content of the stock solution ($3.85 \pm 0.18\%$, Table 1), the estimated solid content delivered per treatment was approximately: 0.039% solids (1% v/v), 0.077% solids (2% v/v), 0.193% solids (5% v/v), and 0.385% solids (10% v/v). Foliar sprays were applied at 14-day intervals using a manual sprayer calibrated to deliver a specific volume to promote leaf runoff without waterlogging the substratum (2.5 mL per plant; 5 sprays). The first application was made 14 days after sowing (DAS),

Table 1 Biochemical and macro- and micronutrient analysis of the aqueous extract of *Kappaphycus alvarezii* applied to barley plants (*Hordeum vulgare* L.), from seaweed biomass produced during autumn, in Palhoça, Santa Catarina, Southern Brazil

Biochemical and Nutrient Analysis	Mean ± SD
Total Solid Content (%)	3.85 ± 0.18
pH	6.57 ± 0.01
Conductivity (mS cm^{-1})	60.56 ± 14.45
Total Carbohydrate (mg g^{-1})	4.96 ± 1.60
Total Soluble Sugar (mg g^{-1})	1.79 ± 0.76
Total Starch (mg g^{-1})	5.51 ± 1.17
Total Phenolic content (mg $(100\ g)^{-1}$)	16.78 ± 5.69
Total Protein (mg g^{-1})	29.38 ± 16.65
Total Amino Acid (mg $(100\ g)^{-1}$)	3.23 ± 1.61
Total Lipid (%)	3.73 ± 1.21
Organic Carbon (%)	0.80 ± 0.91
N (%)	ND
P (%)	0.01 ± 0.00
K (%)	1.62 ± 0.29
Ca (%)	0.01 ± 0.00
Mg (%)	0.03 ± 0.01
S (%)	0.05 ± 0.02
Bo (mg kg^{-1})	1.18 ± 0.55
Cu (mg kg^{-1})	0.08 ± 0.04
Fe (mg kg^{-1})	5.29 ± 2.11
Mn (mg kg^{-1})	0.39 ± 0.36
Na (mg kg^{-1})	2470.40 ± 845.84
Zn (mg kg^{-1})	0.59 ± 0.31

ND Not detected.

corresponding to the tillering stage, and the final application at 74 DAS, corresponding to the end of the vegetative stage, and beginning of heading (i.e., a total of 5 applications). After each application, the plants were randomly rearranged in the greenhouse.

Parameters analyzed

The morphological and yield parameters evaluated were plant height (PH – cm), measured from the base of the stem to the tip of the longest leaf blade; number of total tillers per pot (TTP), number of total spikes per pot (TSP), total number of kernels per pot (NKP), average number of kernels per spike (NKS), and the grain set (i.e., percentage of florets that turned into kernels – GS) as a percentage. At 74 DAS, most plants had initiated the booting process; tissue samples were collected from the third leaf below the flag leaf for biochemical analyses. These included chlorophyll *a*, chlorophyll *b*, total chlorophyll (TChl), and total carotenoids (TCN), as well as primary metabolites including totals of protein (TP), carbohydrates (TC), and amino acids (TAA).

Additionally, the influence of the dilution of seaweed aqueous extract on grain reserve accumulation in the kernels produced (filial generation – F1) was assessed using thousand-kernel weight (TKW) and a germination index (G%), both following standardized protocols (Brasil 2018). To evaluate G%, the germination of 200 kernels from each treatment was assessed using a Germitest paper roll assay (ProLab, 28 × 38 cm, 65 g m^{-2} grammage) moistened with water equivalent to 2.5 times the weight of the paper. Kernels were arranged in four replicates of 50, incubated in a germination chamber at 23 °C and 12 h/12 h light/dark, for 8 days, after which the germination percentage was quantified. For biochemical analysis, a composite kernel sample from each treatment was ground into flour using a knife mill, sieved (50 mesh), and analyzed for primary metabolites: total soluble sugars (F1-TSS), total starch (F1-TS), total protein (F1-TP), and total amino acids (F1-TAA).

Chlorophyll and total carotenoids

For the analysis of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (TChl), and total carotenoids (TCN), 100 mg of leaf samples were incubated in a water bath with 7 mL of dimethyl sulfoxide (DMSO) for 2 h at 65 °C. The extract was recovered by filtration and adjusted to a final volume of 10 mL with DMSO, following the protocol of Hiscox and Israelstam (1979). The absorbance values at 480, 649, and 665 nm were measured and the concentrations were calculated using the formulae provided by Wellburn (1994):

$$\text{Chl}a = \left[12.19 * \left(A_{665}\right) - 3.45 * \left(A_{649}\right)\right]$$
$$\text{Chl}b = \left[21.99 * \left(A_{649}\right) - 5.32 * \left(A_{665}\right)\right]$$
$$\text{TCN} = [1{,}000 * \left(A_{480}\right) - 2.14 * (\text{Chl}a) - 70.16 * (\text{Chl}b)]/220.$$

Total carbohydrate

Total carbohydrate (TC) was determined only for the leaf sample, using a method adapted from DuBois et al. (1956). Briefly, a 50 mg sample of pooled leaves (n = 3) for each treatment was mixed with 20 mL of distilled water, centrifuged (10 min, 4000 rpm), and 2 mL of the supernatant was collected. To this, 50 µL of 80% phenol and 5 mL concentrated sulfuric acid were added. After 10 min the mixture was vortexed and incubated in a water bath (30 °C, 15 min). Absorbance was measured at 490 nm. A calibration curve was prepared using galactose (Sigma-Aldrich, USA) at concentrations from 7.80–500 µg mL^{-1} ($y = 0.0026x$, $R^2 = 0.999$) and the levels were expressed in mg of galactose equivalent per g of dry weight (mg g^{-1}).

Total protein

The total protein (TP) content was determined using the Bradford (1976) method with some adaptations. Briefly, 200 mg of the leaves or flour samples was extracted with 10 mL phosphate-buffered saline (PBS), pH 7, vortexed, allowed to stand for 15 min, and centrifuged (10 min, 4000 rpm). An aliquot of 100 µL of the supernatant was mixed with 5 mL of a 1:5 dilution of the Bradford reagent in water and allowed to stand for 5 min. The absorbance was measured at 595 nm using a microplate reader. A calibration curve was prepared using bovine serum albumin (Sigma-Aldrich) at concentrations ranging from 3.90 to 250.0 µg mL^{-1} ($y = 0.0032x$, $R^2 = 0.977$). The results were converted to mg g^{-1} of dry weight.

Total amino acid

The total amino acids (TAA) was determined using a method adapted from Silveira and Furlong (2007). To each 100 mg of macerated leaves or flour, 2 mL of water was added, vortexed, and centrifuged (4000 rpm, 10 min). A 1 mL aliquot of the supernatant was collected and 3 mL of a 0.2% ninhydrin solution in sodium phosphate buffer (pH 7.0) was added. After stirring, the mixture was placed in a water bath at 100 °C for 30 min. After cooling to room temperature, the absorbance of the reaction was read at 570 nm. An external calibration curve was produced with proline (Sigma-Aldrich) at concentrations ranging from 1.95 to 500 µg mL^{-1} ($y = 0.0018x$, $R^2 = 0.996$). The results were converted to mg g^{-1} of dry weight.

Total soluble sugars and total starch

Total soluble sugars (TSS) and total starch (TS) were analyzed only in the kernels. The TSS were determined using a method adapted from Umbreit and Burris (1964). Briefly, 50 mg of the kernel flour was extracted with 2 mL of methanol, chloroform, and water (MCW, 12:5:3) solution. The mixture was centrifuged (10 min, 4000 rpm) and the supernatant was collected. A second extraction was performed on the pellet and the supernatants were combined. To the mixture were added 1 mL of chloroform and 1.5 mL of water. After stirring and definition of bi-phases, 1 mL of the aqueous phase was collected and to this portion were added 2 mL of 0.2% anthrone in sulfuric acid, followed by further vortexing. The solution was heated in a water bath (100 °C) for 3 min and then cooled to room temperature. Absorbance was measured at 630 nm. Glucose (Sigma-Aldrich) was used to generate a calibration curve with concentrations ranging from 7.8 to 500 µg mL^{-1} ($y = 0.0018x$, $R^2 = 0.992$). The results were expressed in mg as the equivalent amount of glucose per gram of dry weight (mg g^{-1}).

The TS was determined using the same method adapted from Umbreit and Burris (1964). The remaining pellet from the TSS analysis was used for extraction with 2 mL of 30% perchloric acid, centrifuged (10 min, 4000 rpm), and the supernatant was collected. This extraction was repeated and the supernatants were combined. To a 1 mL aliquot, 2 mL of 0.2% anthrone in sulfuric acid was added and the mixture was vortexed. The reaction was heated in a water bath (100 °C, 3 min) and cooled to room temperature. Absorbance was measured at 630 nm. The starch content was calculated from an external calibration curve at concentrations ranging from 1.9 to 500 µg mL^{-1} ($y = 0.0016x$, $R^2 = 0.992$), and the levels were expressed in milligrams of starch equivalent per gram of dry weight (mg g^{-1}).

Statistical analysis

The morphological and productivity variables were performed in eight replicates ($n = 8$) per treatment, as average per pot (PH and NKS) and total per pot (TTP, TSP, NKP). Furthermore, for kernel analysis, a pooled sample of the 8 pots per treatment was used: thousand-kernel weight (TKW) was performed in eight replicates ($n = 8$), while the germination index (G%) of harvested kernels was performed in four replicates ($n = 4$). For the biochemical analyses, composite samples (leaves or kernel flour) were pooled from all eight pots per treatment to ensure representativeness and reduce analytical costs, with technical triplicates ($n = 3$) to account for analytical variability. All results are expressed as mean ± standard deviation (SD).

The extract concentrations were analyzed as categorical variables using the non-parametric Kruskal–Wallis test, with post-hoc comparisons using Dunn's test with Bonferroni adjustment for multiple comparisons ($p_{holm} < 0.05$), performed with the rstatix package (Kassambara 2023) in RStudio software (version 2023.06.0). Graphical representations were generated using the ggplot2 package (Wickham 2016). The parameters were also analyzed using principal component analysis (PCA), employing the singular value decomposition (SVD) algorithm, using Unscrambler X software (v. 10.4).

Results

Morphological and yield parameters

The application of the aqueous extract of *K. alvarezii* via foliar spray to barley resulted in no significant changes to key vegetative morphological parameters across the tested concentrations (0–10% v/v). Specifically, PH ranged from 87.43 ± 8.33 cm in the control to 91.13 ± 3.18 cm at the 1% concentration ($\chi^2(4) = 1.343$, $p = 0.854$). TTP values

varied between 39.4 ± 4.34 (1%) and 52.5 ± 16.03 (control) ($\chi^2(4) = 9.419$, $p = 0.051$), while TSP ranged from 35.13 ± 6.06 (5%) to 44.25 ± 13.11 (control) ($\chi^2(4) = 4.773$, $p = 0.311$). Similarly, the extract did not significantly alter the numerical yield components: total kernels per pot (NKP) ranged from 550.63 ± 79.02 (10%) to 581.00 ± 86.42 (control) ($\chi^2(4) = 0.918$, $p = 0.922$), and average kernels per spike (NKS) varied between 15.23 ± 3.92 (control) and 17.21 ± 1.49 (1%) ($\chi^2(4) = 2.297$, $p = 0.681$). Complete graphical representations of these non-significant parameters are available in supplementary material (Figs. S1 and S2).

In contrast, the extract induced significant, concentration-dependent improvements in grain quality parameters. Grain set (GS) was significantly enhanced ($\chi^2(4) = 17.515$, $p = 0.002$), with the 1% treatment showing an 18.24% increase (81.30 ± 4.09%) compared to the control (68.76 ± 7.74%). Thousand-kernel weight (TKW) also exhibited a significant positive dose–response ($\chi^2(4) = 12.143$, $p = 0.016$), with the highest values recorded at 5% (53.37 ± 0.92 g) and 10% (53.30 ± 1.50 g), representing an increase of approximately 4.2% over the control (51.13 ± 1.39 g) (Fig. 1).

A principal component analysis (PCA, Fig. 2) was applied to morphological and yield data to effectively capture the treatment-induced variations. The first two principal components (PCs) accounted for 94% of the total variance (PC1: 78%, PC2: 16%). The projection of the data on the PC1-PC2 plane revealed a clear segregation among the treatments. The control (0%) was distinctly isolated in the positive extreme of PC1 +, while the extract treatments formed separate clusters on PC1-. The parameters plant height (PH), number of kernels per spike (NKS), and grain set (GS) were grouped to the treatment 1%, on PC1- and PC2-, while thousand-kernel weight (TKW) was grouped to treatments 2, 5, and 10%, on PC1- and PC2 + (Fig. 2).

Biochemical composition of plants

Regarding photosynthetic pigments (Chl *a*, Chl *b*, TChl, and TCN), the Kruskal–Wallis test revealed a significant overall effect of extract concentration ($\chi^2(4) = 13.500$; $p = 0.009$). However, after Bonferroni correction for multiple comparisons, only the 5% concentration differed significantly from the control ($p < 0.05$), while intermediate concentrations (1, 2, and 10%) did not reach statistical significance in pairwise comparisons (Fig. 3). The lowest values occurred at 5% concentration, a reduction of approximately 50.5% as compared to control ($\chi^2(4) = 13.500$; $p = 0.009$) for all pigments.

Regarding the primary metabolites (Fig. 4), TC peaked at 5% *Kappaphycus* extract concentration (27.20 ± 0.25 mg g^{-1}), which was significantly higher than the 10% treatment (20.33 ± 1.30 mg g^{-1}) but did not differ statistically from the control or other concentrations ($\chi^2(4) = 11.733$; $p = 0.019$). TP showed no significant differences across treatments ($\chi^2(4) = 7.138$; $p = 0.129$), with values ranging from 0.08 ± 0.03 mg g^{-1} at 1% to 0.25 ± 0.10 mg g^{-1} at 5% concentration. TAA presented the highest value at 1% concentration (58.12 ± 1.34 mg g^{-1}), with an increase of 47.44% compared to the control (39.42 ± 1.91 mg g^{-1}; $\chi^2(4) = 13.500$; $p = 0.009$).

When using PCA on biochemical parameters, a total variance of 98% was found (PC1: 58%, PC2: 24%). Two distinct clusters were observed on PC1- quadrant, with 5% and 10%

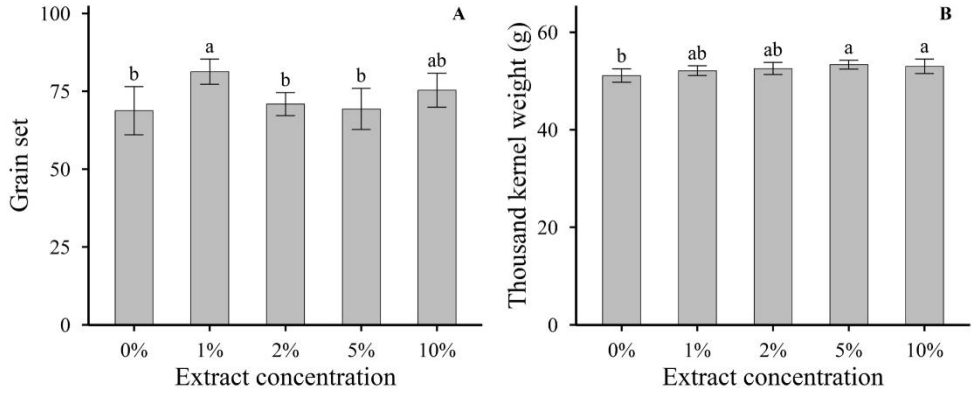

Fig. 1 Yield components of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses diluted in water (control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil. A – grain set (GS); B – thousand kernel weight (TKW – g). Data are presented as mean + standard deviation. Values followed by different letters differ significantly for Dunn's test with Holm-Bonferroni adjustment ($p < 0.05$)

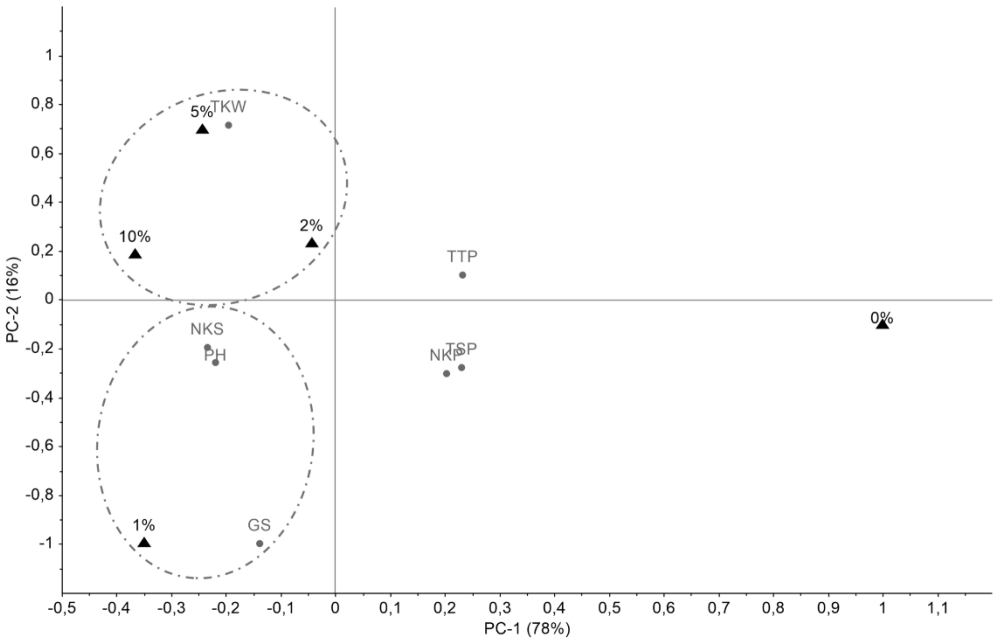

Fig. 2 Principal component analysis (PC1 and PC2) calculated from the data of plant height (PH), total tillers per pot (TTP), total spikes per pot (TSP), total kernels number per pot (NKP), number of kernels per spike (NKS), grain set (GS), and thousand-kernel weight (TKW), of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii*, at different doses (control, 1, 2, 5, and 10% v/v). The seaweed raw materials were cultivated in Santa Catarina, Brazil

grouping with total protein (TP), and 2% grouping with total amino acids (TAA) and total carbohydrates (TC) (Fig. 5).

Germination and biochemical composition of kernels

The germination index (G%) of grains produced by treated plants was consistently high (98.5–100%) and not significantly different across all treatments ($\chi^2(4) = 5.146$; $p = 0.273$). However, all the biochemical components of the kernels were significantly affected (Fig. 6). Total starch content (F1-TS) presented the highest value at 5% concentration (21.79 ± 0.25 mg g^{-1}), differing statistically from the control (18.26 ± 0.23 mg g^{-1}), representing an increase of 19.33% of TS ($\chi^2(4) = 12.033$; $p = 0.017$). Conversely, total soluble sugar content (F1-TSS) was significantly lower at the 5% concentration (161.54 ± 2.64 mg g^{-1}) compared to the control (188.01 ± 3.24 mg g^{-1}), a downgrade of 14.08% ($\chi^2(4) = 10.867$; $p = 0.028$). The total protein (F1-TP) increased in 10% concentration (1.82 ± 0.06 mg g^{-1}), differing statistically from the control (1.65 ± 0.06 mg g^{-1}), representing an increase of 10.30% ($\chi^2(4) = 9.951$; $p = 0.041$). The amino acid content (F1-TAA) was higher in 1% and 2% concentrations (17.30 ± 0.77 and 17.70 ± 0.16 mg g^{-1}, respectively), resulting in a significant increase of 54.87% compared to the control (11.30 ± 0.75 mg g^{-1}; $\chi^2(4) = 12.300$; $p = 0.015$).

When employing PCA on the kernel parameters, a total variance of 100% was observed between PC1 (61%), PC2 (35%), and PC3 (4%) (Fig. 7). Four distinct clusters were observed across three quadrants, with the 10% treatment grouping with F1-TSS on PC1 + and PC2 +; the 1% treatment grouping with F1-TS and 2% grouping with F1-TP and F1-TAA, on PC1- and PC2 +; and 5% grouping with germination index (G%).

Discussion

The applications of the aqueous extracts of *K. alvarezii* elicited a concentration-dependent response in barley, significantly altering key yield components and biochemical profiles, while leaving basic plant architecture largely unchanged.

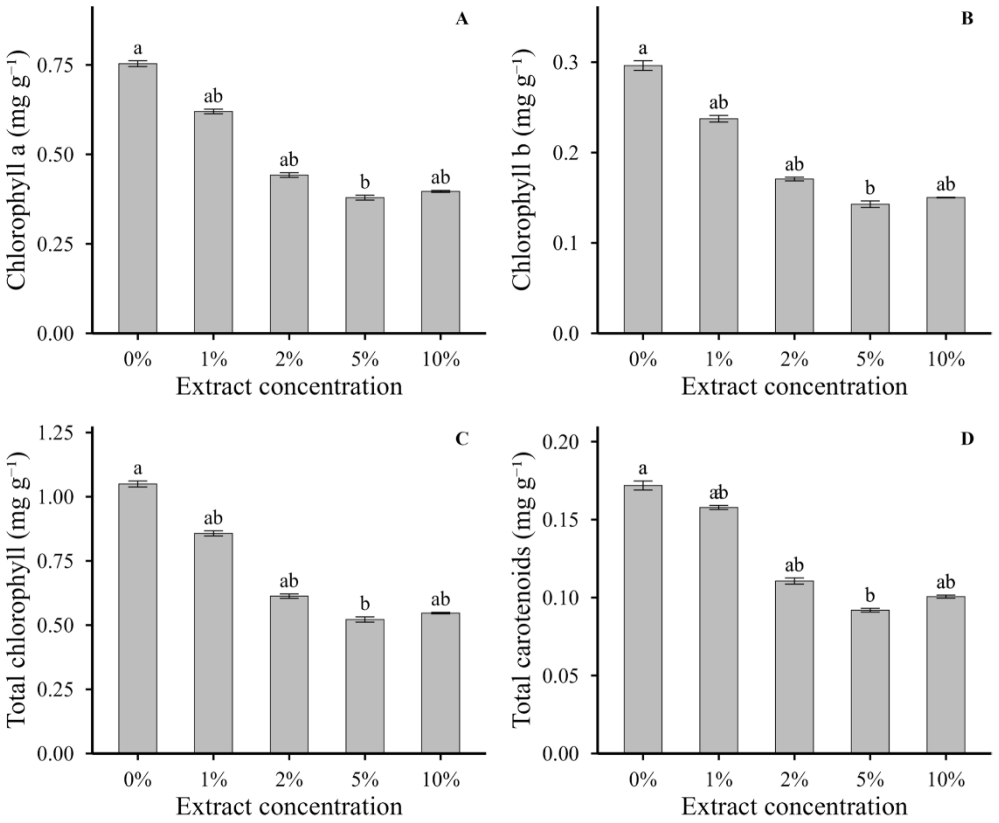

Fig. 3 Leaf pigments (mg g^{-1}) of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses diluted in water (control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil. A – chlorophyll *a*; B – chlorophyll *b*; C – total chlorophyll; D – total carotenoids (TCN). Values followed by different letters differ significantly for the Dunn's test with Holm-Bonferroni adjustment ($p < 0.05$)

The extract had a minimal direct impact on vegetative growth under the controlled greenhouse environment. The findings suggested that the commercial potting medium pre-fertilized with NPK (10:10:10) at 5 g per pot provided sufficient baseline nutrition to support optimal vegetative development of the barley plants in the greenhouse, potentially diminishing the extract's additive effects on these traits. However, there have been more consistent, positive responses reported in other cereals, e.g., in wheat, rice, and maize, foliar applications of *K. alvarezii* extracts have been shown to promote growth metrics such as height, biomass, and tillering, often attributed to bioactive compounds such as cytokinins, auxins, and polysaccharides that enhance cell division, nutrient mobilization and root architecture (Trivedi et al. 2023; Fatima and Al-Yasari 2024). Furthermore, the use of a single NPK regime (10:10:10 at 5 g per pot) precludes assessment of the extract's performance under reduced or variable fertilization inputs, a scenario increasingly relevant to sustainable barley production and one in which seaweed-based biostimulants have shown stronger additive effects (Cozzolino et al. 2021). Future experiments should test this extract under a gradient of nitrogen availability to disentangle nutritional interactions from direct biostimulant effects.

An additional limitation related to the experimental setup concerns the use of 10-L pots, whose restricted soil volume may have constrained root expansion and limited the expression of root-promoting effects commonly reported for *K. alvarezii* extracts, such as enhanced root length, lateral root density, and root biomass (Trivedi et al. 2018; Kumar et al.

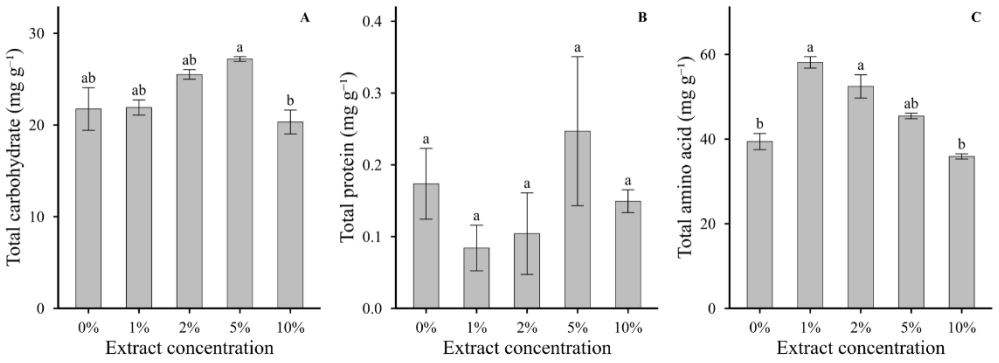

Fig. 4 Biochemical parameters (mg g^{-1}) of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses diluted in water (0, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil. A – total carbohydrates (TC); B – total protein (TP); C – total amino acid (TAA). Values followed by different letters differ significantly for the Dunn's test with Holm-Bonferroni adjustment ($p < 0.05$)

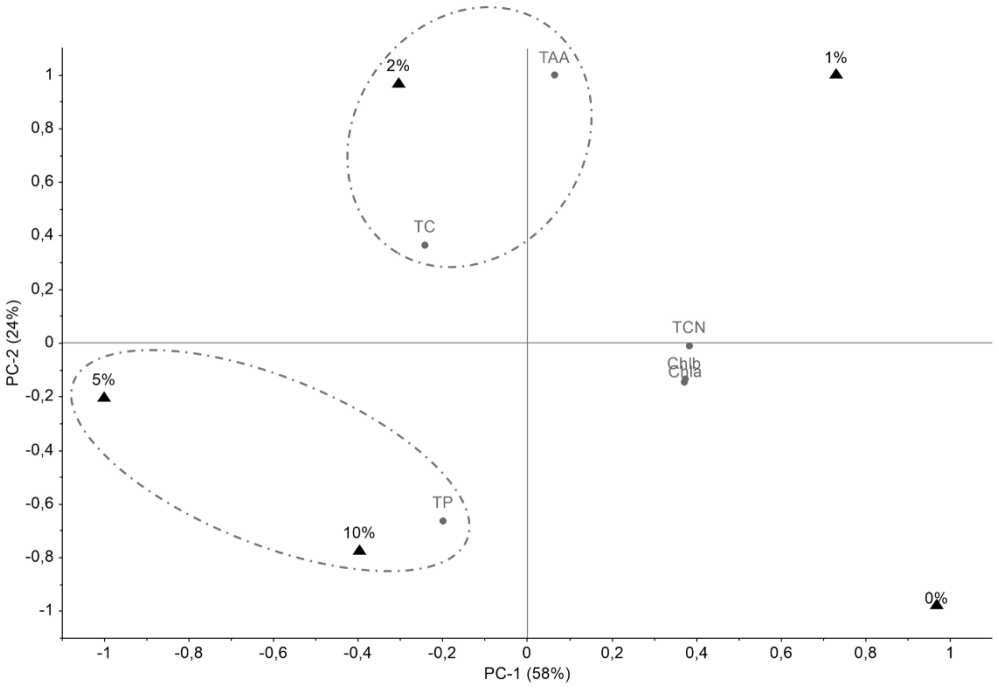

Fig. 5 Principal component analysis (PC1 and PC2) calculated from the dataset of plant biochemical components chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total carotenoids (TCN), total carbohydrates (TC), total proteins (TP), and total amino acids (TAA), of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses (control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil

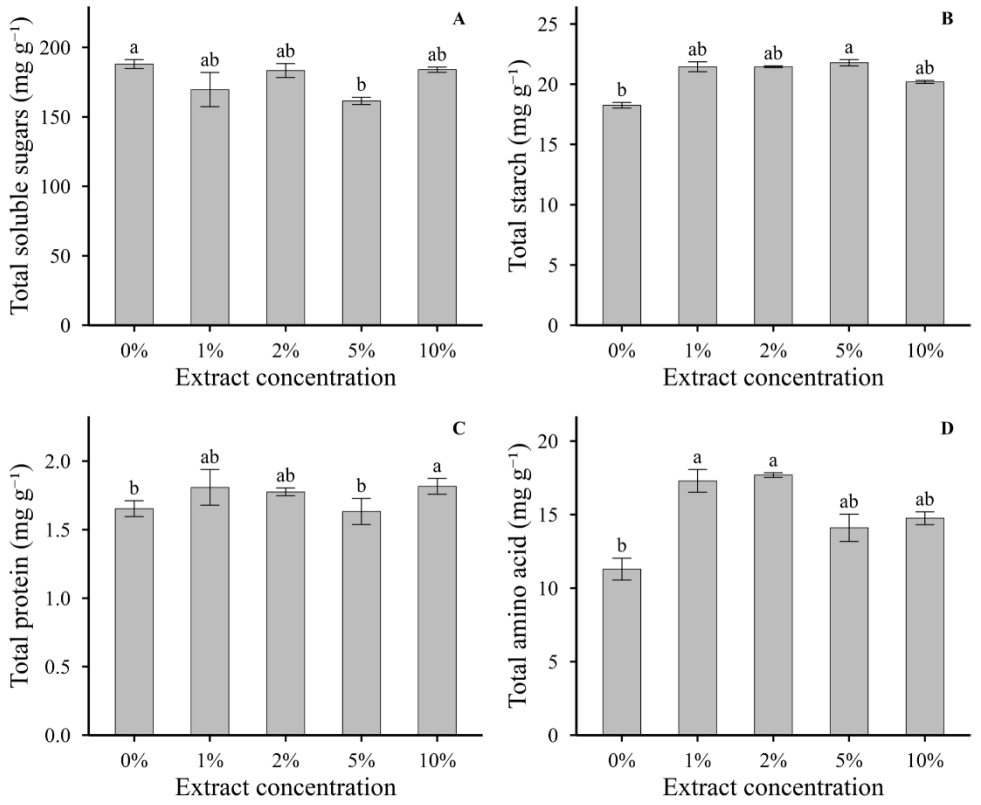

Fig. 6 Biochemical parameters (mg g^{-1}) of kernels produced by barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses (control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil. A – total soluble sugars (TSS); B – total starch (TS); C – total protein (TP); D – total amino acid (TAA). Values followed by different letters differ significantly for the Dunn's test with Holm-Bonferroni adjustment ($p < 0.05$)

2020). Root morphology was not assessed in the current study, and this represents both a limitation and an important avenue for future greenhouse and field investigations.

A review of the literature specific to barley revealed highly inconsistent effects of seaweed-based biostimulants on morphology, likely due to the wide variation in experimental conditions, extract formulations, and application protocols among the studies (Table 2). Multi-year field trials have documented subtle improvements in grain yield, even in the absence of significant alterations to plant height, tiller density, or spike formation (Taylor et al. 1990; Ashworth et al. 2025). When morphological changes do occur, they are context-dependent, modulated by variables such as formulation (e.g., extract purity and concentration), application timing (e.g., tillering vs. heading stages), environmental factors (e.g., soil type, climate variability), and historical agronomic practices (e.g., prior fertilizer regimes) (Szczepanek 2017; Hřivna et al. 2024).

For example, plant height in barley often remains stable across foliar applications of various seaweed extracts in non-stressed, well-fertilized settings, with increases primarily documented under abiotic stressors such as drought or salinity, or when the extracts were applied via soil drenching to target root zones more effectively (Szczepanek 2017). Tiller and spike numbers are similarly variable, frequently influenced by weather patterns that overshadow foliar-induced responses; in some cases, enhancements require synergistic practices, such as combining seaweed extracts with reduced nitrogen applications, or seed priming to optimize resource partitioning (Cozzolino et al. 2021; Hřivna et al. 2024).

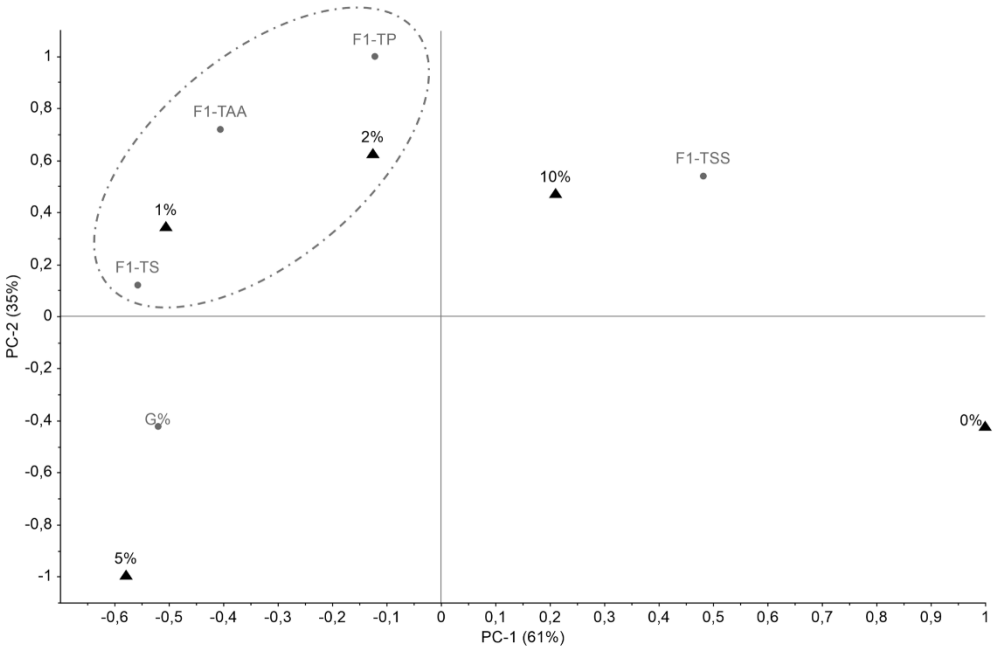

Fig. 7 Principal component analysis (PC1 and PC2) calculated from the dataset of kernel germination (G%) and biochemical components: total starch (F1-TS), total soluble sugars (TSS) total protein (TP), and total amino acids (TAA), of barley plants treated with foliar application of aqueous extract of *K. alvarezii* at different doses (control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil

Under conditions of high soil fertility, as simulated here with NPK supplementation, vegetative and partitioning traits may reach a physiological plateau, limiting the ability of the *Kappaphycus* extract to elicit measurable changes (Ashworth et al. 2025). This could also stem from the inherent genetic traits of specific varieties of barley, which may confer less responsiveness to exogenous signals as compared to other cereals, or the experimental design's absence of imposed stressors. It is also important to note that the observational nature of this study does not allow resolution of the molecular and physiological mechanisms underlying the responses observed, including the potential roles of bioactive compounds such as cytokinins, betaines, and sulfated polysaccharides. Elucidating these mechanistic pathways in barley represents a priority for future research. Future research should aim to decouple these effects by testing particular extracts under varying nutrient regimes (e.g., reduced NPK), or in conjunction with abiotic stressors, to clarify whether its primary role is corrective under stress or optimizing under non-limiting conditions. Regarding the application regime adopted here (five foliar sprays at 14-day intervals from 14 to 74 DAS), it should be acknowledged that timing, frequency, and phenological stage of application can strongly modulate biostimulant performance (Szczepanek 2017; Hřivna et al. 2024). Applications initiated closer to the reproductive stage, such as at flag leaf emergence or anthesis, may produce different or complementary effects on yield components and should be explored in future studies.

The influence of concentrations of the specific *Kappaphycus* extract tested here was selective, enhancing the quality of kernel production rather than its quantity. While it did not affect the number of kernels per pot, or per spike, it significantly improved grain set and thousand-kernel weight (TKW). The increase in grain set at low concentrations (1–2%) suggested a promotion of reproductive efficiency and pollination success, likely through improved resource allocation during flowering. The significant increase in grain set in barley plants can be interpreted through the physiological critical window of anthesis. This period, encompassing flowering and pollination, is decisive for yield determination in barley, as abiotic stresses often lead to floret abortion and directly limit the final number of grains (Dolferus

Table 2 Comparative overview of reported effects of seaweed-derived extracts on barley (*Hordeum vulgare L.*)

Parameters	Taylor et al. (1990)	El-Sharkawy et al. (2017)	Szczepanek et al. (2018)	Cozzolino et al. (2021)	Goñi et al. (2021)	Hřivna et al. (2024)	Al-Musawi et al. (2025)	Present work
Seaweed	Unspecified marine seaweed (likely *Ascophyllum nodosum* or similar, containing cytokinins)	*Ascophyllum nodosum*	*Ecklonia maxima* (Kelpak)	*Ecklonia maxima*	*Ascophyllum nodosum*	*Ascophyllum nodosum*	*Ascophyllum nodosum*	*Kappaphycus alvarezii*
Extract type	Liquid extract with cytokinins	Commercial liquid extract	Seaweed extract (aqueous)	Seaweed extract (cold cell burst, high auxin:cytokinin ratio)	ANE biostimulant PSI-362	Liquid extract (1% *A. nodosum*)	ANE Goëmar GA142	Aqueous extract
Application method	Foliar spray at 4–5 leaf stage (1 L ha^{-1} or 2.5 × rate)	Foliar (4 kg ha^{-1}, once)	Foliar (2 L ha^{-1} or sequential 1.5 L ha^{-1})	Foliar (3 applications at 3.0 mL L^{-1})	Coated on fertilizer (CAN + S)	Foliar (1 L $há^{-1}$)	Foliar (1 L ha^{-1}, 1:50 dilution)	Foliar (1–10% v/v)
Morphological Results	No significant changes in plant height, tiller number, head length, or maturity; no promotion under optimal or stress conditions	Increased shoot/root dry weight (up to 4.19/3.89 g), tillers (up to 7.44), root length (up to 18.22 cm) under salt (10–15 dS m^{-1})	Root weight + 15–17%; grain/spike + 4%; TGW + 4.3% (sequential application superior; better root development under varied weather)	Height + 4.2%; spikes + 9%; biomass + 15.7%; TKW + 3.9%; kernel diameter + 2.3% (higher at N40/N60, plateau at N40 in warmer year; extract amplifies at mid-N doses)	Yield + 5.57%; biomass + 13.88–16.67% (coating enhances under N/S fertilization; no stress-specific morph changes)	Increased root size (length/volume up to 20%); no change in shoot height; better under dry conditions	Aboveground biomass + 76.82%; spikelets m^{-2} + 75%; TGW + 27%; grains/spike + 15.22% (UI + B superior; genotype-specific, e.g., late-maturing more responsive early-stage)	No significant changes in plant height, total tillers, and total spikes
Biochemical Results	No changes in nutrient uptake or stress-related markers; cytokinin levels did not enhance metabolism	Reduced Na^+/K^+ ratio (to 5.54–7.15); increased proline (up to 0.48 µmol g^{-1} FW), catalase (up to 450 mmol min^{-1} g^{-1} FW); decreased EL (to 72.39%)	P content +; N/P uptake + (enhanced nutrient efficiency)	Grain protein + 24.2% (11.8% vs. 9.5%; exceeds 11.5% at N60; NUE + 17.9%; inverse with N dose)	Nitrate + 17.9–72.2%; NUE + 29.85–60.26%; NR + 24.82%; GS + 30.63% (higher under coated fertilizer)	Enhanced N uptake (up to 15%); improved nutrient efficiency	Grain protein 9.5–11.5% (optimal malting; stable under reduced N)	Leaf amino acids (+ 47,44% at 1%), kernel starch (+ 19.3% at 5%); kernel protein (+ 10.3% at 10%); kernel amino acids (+ 56.6% at 1–2%)

Table 2 (continued)

Parameters	Taylor et al. (1990)	El-Sharkawy et al. (2017)	Szczepanek et al. (2018)	Cozzolino et al. (2021)	Goñi et al. (2021)	Hřivna et al. (2024)	Al-Musawi et al. (2025)	Present work
Yield Results	No increase in grain yield (field trials over 4 years); similar to kinetin application; no edge cases like drought benefits observed	Increased relative yield (up to 75.23%); better under salt stress; no non-stress benefits	Yield + 4% (sequential better; grain quality improved via TGW)	Grain yield + 17.0%; hectolitre mass + 3.8% (higher in warmer year; N40 sufficient with extract, reducing N by 35%; harvest index unaffected)	Yield + 5.57% (via biomass/NUE; no detailed components)	Increased grain yield (up to 10%) and components; better in low-N soils	Grain yield + 71%; malt able fraction + 22% (UI + B; retention > 90%; environment/genotype interactions; GPC optimal)	Increased GS (+ 18.24% at 1%) and TKW (+ 4.2% at 5–10%). No significant changes in kernels per pot and kernels per spike

et al. 2011; Boussora et al. 2019; Kamal et al. 2022). The enhanced grain set suggests that this extract positively influenced this sensitive stage, potentially contributing to patterns observed in yield variables such as NKS (grouped in the PCA with GS), although without altering the total kernel (NKP). This may be mediated by improvements in the plant's nutritional status (Askarnejad et al. 2021), a more favorable hormonal balance for floret fertility (Boden 2016; Marzec and Alqudah 2018), or enhanced resilience to a transient environmental stress during flowering such as a high night temperature (García et al. 2015; supplementary material Fig. S3). Consequently, the application of *K. alvarezii* extract did not increase the potential number of florets/kernels but rather secured a higher success rate in their development into viable grains, highlighting a role in optimizing reproductive efficiency.

In contrast, the higher TKW at moderate-to-high concentrations (5–10%) points to a direct role in enhancing grain filling and reserve accumulation. These findings align with barley-specific studies where the foliar application of *E. maxima* extract (large brown seaweed species native to the cold, nutrient-rich waters) boosted grain number per spike, thousand-grain weight, and overall yield by 8–20%, attributed to enhanced root development, nutrient uptake (N and P), and hormonal signaling (Szczepanek et al. 2018). In broader cereals like maize, *K. alvarezii* extracts have demonstrated similar yield enhancements under non-stress conditions, with optimal responses at concentrations of 5–10% (e.g., 19–36% yield increases), attributed to bioactive compounds including cytokinins, auxins, and polysaccharides that promote cell division, expansion, and stress tolerance (Rabhi et al. 2025). The dose-dependent patterns observed here, favoring low doses for grain set and higher for TKW, echo context-specific responses in field trials, where seaweed extracts often optimize partitioning under non-stress environments by modulating gene expression for nutrient efficiency (Trivedi et al. 2018; Kumar et al. 2020).

Biochemically, chlorophyll is primarily responsible for capturing light energy, which is then converted into chemical energy through photosynthesis. In contrast, carotenoids assist in light absorption and provide essential photoprotection by dissipating excess energy, thus preventing damage to the photosynthetic apparatus (Chen 2015; Nguyễn and Sung 2024). The observed pigment reduction in 5% concentration diverges from typical *K. alvarezii* extract, which generally enhances chlorophyll and carotenoid levels on plants, even under non-stress conditions (e.g., Nivetha et al. 2024; Nunes et al. 2025b).

In contrast, the analysis of total carbohydrate (TC) levels revealed a peak at the 5% concentration of the aqueous extract. Although this value did not significantly differ from the control, it was grouped only with the 2% treatment in the PCA (Fig. 5). TC in plant leaves is essential for energy

storage and metabolic processes (Lal and Bhatla 2023). The accumulation of carbohydrates can modulate photosynthetic rates, where excess may lead to feedback inhibition, underscoring their role in maintaining plant homeostasis (Araya et al. 2006; Khornti 2023), and serving as reserves under stress conditions, such as cold stress (Babazadeh et al. 2023).

Some works demonstrate increases in total or non-structural carbohydrates in barley leaves treated with seaweed extracts. For instance, seaweed extracts enhance non-structural carbohydrates in barley, promoting freezing tolerance by facilitating energy storage and water adjustments under cold stress, with applications leading to multifaceted phytostimulatory effects that boost overall crop resilience and yield (Ali et al. 2021a). In brewing barley subjected to varying nitrogen levels, *A. nodosum* extract increases grain protein and metabolic profiles, indirectly supporting leaf carbohydrate dynamics through reduced nitrogen dependency and enhanced biomass, though direct leaf TC measurements are not always specified (Cozzolino et al. 2021).

Studies with other cereals treated with other extracts of *K. alvarezii* showed notable TC elevations, particularly in maize leaves, where foliar applications of the AgroGain® (commercial biostimulant) at 1 mL L^{-1} increased total soluble sugars by up to ninefold, linked to heightened photosynthetic activity, chlorophyll content, and rhizosphere microbial priming that enhances nutrient utilization and plant vigor (Nivetha et al. 2024). In non-cereal plants, such as basil (*Ocimum basilicum*), the application of a similarly prepared aqueous extract of *K. alvarezii* from southern Brazil also increased plant TC, with a peak at 5–7% concentrations (Nunes et al. 2025b). These patterns suggested that 5% peak in TC may reflect an optimal dose for carbon assimilation in barley, potentially aiding stress resilience and yield, consistent with seaweed's role in sustainable crop production. It is important to note that while both extracts are aqueous preparations of *K. alvarezii* from Santa Catarina, they differ in production scale: the extract used in the present study was produced at an industrial scale (provided by VerdeMar®), whereas the extract in Nunes et al. (2025b) was prepared at a laboratory scale. These differences in processing scale may result in variations in their specific biochemical profiles and, consequently, their bioactivity. Such variations underscore that the bioactivity of algal extracts is not only species-dependent but also influenced by biogeographic factors (Nunes et al. 2025a), seasonal and processing differences (even if the same seaweed), and these are points that need explicit investigation in future comparative studies. It should therefore be emphasized that the effects reported here pertain specifically to an autumn-harvested biomass from Palhoça, Santa Catarina, Brazil, processed at an industrial scale, and may not fully represent the agronomic performance of *K. alvarezii* extracts derived from different origins, seasons, or processing methods.

The application of the *K. alvarezii* extract in this study influenced the levels of total proteins (TP) and total amino acids (TAA) in leaves. While foliar TP showed no significant changes (overall average: 0.15 ± 0.08 mg g^{-1}), it was grouped with the 5% and 10% treatments in the PCA. In contrast, the TAA content in leaves was significantly increased by the 1%, 2%, and 5% concentrations, representing increases of 47.4%, 33.1%, and 15.3%, respectively, over the control (39.42 ± 1.91 mg g^{-1}). Although foliar TP was not significantly influenced by this *K. alvarezii* aqueous extract, the marked increase in TAA corroborates the findings of Goñi et al. (2021), who reported significant increases of approximately 23% in total soluble proteins and free amino acids in barley plants treated with *A. nodosum* extract. This modulation is significant, as amino acids are not only the building blocks of proteins but also act as crucial signaling molecules for nitrogen distribution, influencing plant architecture and stress responses (Guo et al. 2021).

In turn, kernels exhibited significant biochemical alterations following foliar application of *K. alvarezii* aqueous extract, with implications for grain quality and malting performance. The elevation in total starch content (F1-TS) at 5% concentration, representing a 19.3% increase over the control, underscores enhanced reserve accumulation, as starch constitutes the predominant fermentable substrate in malting processes (Schepper and Courtin 2024). This augmentation predicts improved extract yields during mashing, a paramount criterion for malt economic viability and quality (Evans et al. 2005; Nehra et al. 2024). Furthermore, the observed reduction in total soluble sugars (F1-TSS) at the same concentration (14.1% decrease) reflects a metabolic pivot favoring starch polymerization over transient sugar retention during grain maturation (Shewry et al. 2002). Such a profile confers kernel stability, facilitating controlled saccharide release in malting rather than premature availability (Briggs 1998).

The total protein (F1-TP) increased 10.3% in kernels at 10% treatment. While this rise is moderate, it could enhance attributes like foam retention and yeast nutrition but must be balanced to prevent problems in processing (Nehra et al. 2024). In turn, total amino acids (F1-TAA) had a substantial increment of 54.87% over the control at 1% and 2%. It represents the extract efficacy, as free amino nitrogen (FAN) is vital for yeast assimilability, ensuring fermentation integrity and mitigating off-flavor risks like hydrogen sulfide (Lekkas et al. 2005). It is important to highlight that these significant improvements in grain biochemistry occurred without any negative impact on the grain's viability. The germination index (G%) remained consistently high (98.5–100%) across all treatments, indicating that the extract did not induce seed dormancy or damage. This is a critical practical advantage, as high and uniform germination is a non-negotiable prerequisite for the malting process itself. Despite the scarcity of

literature on the effects of seaweed extracts on barley grain biochemistry, the available evidence points to significant yet species-specific improvements. For example, as summarized in Table 2, a study with *A. nodosum* extract on brewing barley under varying nitrogen regimes demonstrated a substantial 24.2% increase in grain protein (Cozzolino et al. 2021). While direct studies with *K. alvarezii* on barley grains are lacking, parallel research in wheat suggests this species can also modulate key grain components like starch and protein (Layek et al. 2015). These findings, together with our results on protein and amino acids, underscore that biochemical enhancements are possible, but their magnitude and nature are likely mediated by the distinct bioactive profiles of different algal extracts.

These quality enhancements carry significant potential value for the malting barley industry. The observed increases in thousand-kernel weight (+4.2%) and grain protein (+10.3%) directly contribute to improved malt extract potential and brewing quality, while the substantial rise in free amino acids (+56.6%) enhances free amino nitrogen (FAN) availability, a critical factor for efficient yeast fermentation and consistent beer production. Although a full cost–benefit analysis was beyond the scope of this study, these improvements suggest that strategic application of this extract could translate into higher-quality grain with premium market value. Future field-scale studies should include economic assessments to quantify the return on investment for growers and the malting industry.

Conclusion

This study demonstrated that the primary agronomic value of the aqueous extract of *K. alvarezii* for barley lies in its specific capacity to enhance grain quality for the brewing industry, rather than in promoting vegetative growth, a pattern that contrasts with the more consistent growth stimulation reported in cereals like maize and wheat. It is important to note, however, that the absence of vegetative growth stimulation observed under these conditions (well-fertilized, pot-grown, and greenhouse-controlled) should not be interpreted as evidence of a general lack of vegetative responsiveness in barley to *K. alvarezii* extracts. This outcome is condition-specific and may differ under reduced fertility, field conditions, or abiotic stress. These findings suggest the extract's potential for complementary use, targeting quality traits specifically. The significant increases in kernel starch, protein, free amino acids, and thousand-kernel weight, achieved without compromising germination, represent a direct strategy to improve key malting parameters.

However, the context-dependent nature of the responses, particularly the selective effects on reproductive over vegetative traits, and the concentration-specific reduction in leaf pigments, underscores the importance of species-specific mechanistic investigation to fully interpret these patterns. Future research should focus on elucidating the molecular pathways triggered in barley, validating these quality enhancements under diverse field conditions, and exploring the synergistic use of this extract with reduced fertilizer regimes or in combination with other seaweed extracts to fully integrate it into sustainable malting barley production systems. Finally, these results highlight that different crops may respond distinctly to the same biostimulant input, reinforcing that the optimization of extract type, nutrient regime, and application strategy must be conducted on a crop-specific basis to fully unlock the potential of *K. alvarezii* as a sustainable agricultural input in diverse agroecosystems.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10811-026-03883-z.

Acknowledgements We would like to thank VerdeMar Ltda. for the kind provision of the commercial K. alvarezii aqueous extract for this study.

Author's contributions F.S.D., E.R.O., and M.M. contributed to the study conception and design. Material preparation and data collection were performed by F.S.D., E.R.O., G.Z.A., L.V.P.M., L.R.B.S., and I.F.A.. Analysis was performed by F.S.D., E.R.O., A.N., and A.R.S.. The first draft of the manuscript was written by F.S.D., and all authors commented on previous versions of the manuscript. Project administration and funding acquisition were performed by M.M. and S.M..

Funding The Article Processing Charge (APC) for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614). Research was supported by grant 88887.696139/2022—00 to F.S.D and 88887.667199/2022—00 to L.V.P.M from Coordination for the Improvement of Higher Education Personnel (CAPES); by grants 306495/2023—6 (M.M.), 303956/2023—2 (E.R.O.), 314977/2025—2 (A.R.S.) from National Council for Scientific and Technological Development, Brazil (CNPq); by grant 2023/03886—1 to A.N. from São Paulo Research Foundation (FAPESP).

Data availability The results of the analyses conducted for this manuscript are available and can be provided to researchers upon request. Interested parties are invited to contact the authors of the manuscript for access to the data.

Declarations

Competing interests The authors declare no competing interests.

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6. Discussão Geral

A crescente demanda por sistemas agrícolas mais resilientes e sustentáveis, impulsionada pelos impactos negativos do uso intensivo de insumos químicos sintéticos e agravada pelas projeções de mudanças climáticas (Pretty et al., 2018; Raza et al., 2020; Zandalinas et al., 2021), tem impulsionado o desenvolvimento de bioinsumos de base biológica capazes de conciliar produtividade e conservação ambiental. Nesse cenário, os extratos de macroalgas marinhas emergem como uma das alternativas mais promissoras, dada sua capacidade de modular múltiplas vias fisiológicas nas plantas e de serem produzidos a partir de biomassa cultivável em larga escala (du Jardin, 2015; Ali, Ramsubhag & Jayaraman, 2021a; Shukla et al., 2025). A macroalga vermelha *Kappaphycus alvarezii* ocupa, nesse contexto, uma posição estratégica singular no Brasil: sua recente aprovação para cultivo comercial (Brasil, 2020b) e a consolidação de Santa Catarina como principal polo produtor nacional (Santos, 2024; Hayashi et al., 2024) criaram as condições necessárias para que a ciência avance da observação empírica de efeitos bioestimulantes à caracterização mecanística e ao respaldo técnico para o registro de produtos. A presente tese foi concebida precisamente nessa lacuna, tendo integrado quatro capítulos complementares que cobrem desde a composição bioquímica sazonal dos extratos e de seus resíduos até a validação agronômica do potencial bioestimulante na cultura da cevada (*Hordeum vulgare* L.).

6.1. Sazonalidade como determinante da qualidade do bioestimulante

O primeiro eixo central desta tese, a caracterização bioquímica sazonal do extrato aquoso comercial (Capítulo 1), revelou que a composição do produto está longe de ser estática, sendo profundamente modulada pelo período do ano. As correlações negativas e estatisticamente significativas entre a temperatura da superfície do mar (SST) e as concentrações de aminoácidos totais (TAA, $\rho = -0{,}76$), amido total (TS, $\rho = -0{,}67$), açúcares solúveis (TSS, $\rho = -0{,}62$), fenóis totais (TPC, $\rho = -0{,}57$) e carboidratos totais (TC, $\rho = -0{,}52$), bem como dos nutrientes sulfato (S, $\rho = -0{,}73$) e potássio (K, $\rho = -0{,}62$), indicam que as estações frias são aquelas em que a alga acumula uma maior densidade de metabólitos bioativos e nutricionalmente relevantes. Esse padrão encontra suporte na literatura disponível para outras espécies de macroalgas, que documentam acúmulo sazonal de compostos nitrogenados e fenólicos em resposta à redução de temperatura e à menor irradiância do inverno (Deyab, 2011;

Khairy & El-Shafay, 2013; Kumar et al., 2021). Para *K. alvarezii* cultivada no Brasil, Araújo et al. (2022) também registraram variação sazonal nas propriedades nutricionais e antioxidantes de diferentes linhagens, reforçando que a sazonalidade é um fator inerente à espécie e não um artefato de processo. Os dados do Capítulo 1 corroboram esse entendimento ao demonstrá-lo, de forma inédita para o Atlântico Sudoeste subtropical, em extratos comerciais já processados, o que é diretamente relevante para fins de controle de qualidade e registro de produtos.

Dentre os macronutrientes, o potássio se mostrou o mais abundante (0,97% a 1,82%), em consonância com os dados de Zodape et al. (2008), Rathore et al. (2009) e Layek et al. (2015), que reportaram concentrações entre 16 e 33 g/L em extratos aquosos de *K. alvarezii*. Embora os demais macronutrientes (N, P, Ca, Mg) tenham sido detectados em concentrações muito baixas — insuficientes para suprir as necessidades nutricionais das plantas —, o teor expressivo de potássio posiciona o extrato como potencial fonte desse nutriente em cultivos específicos, como já demonstrado por Karthikeyan & Shanmugam (2017) em experimentos com cana-de-açúcar. A análise de componentes principais (PCA) consolidou esses achados ao discriminar claramente dois grupos sazonais de amostras de *Kappaphycus alvarezii* consoante às estações de colheita, i.e., verão e inverno, ao evidenciar que a variabilidade entre os locais de produção (Palhoça e Florianópolis) foi secundária em relação à variabilidade temporal, o que sugere razoável homogeneidade nos processos produtivos entre os fornecedores avaliados.

6.2. O resíduo como ativo estratégico: rumo à biorrefinaria em cascata

O segundo eixo desta tese, a caracterização do resíduo sólido da produção (Capítulo 2), é, do ponto de vista da inovação tecnológica, possivelmente o mais original entre os capítulos individuais. O resíduo retém valores elevados de cinzas (25,37 a 31,87%, peso seco), com picos de Ca, Mg e S na primavera, e apresentou rendimento expressivo de carragenana residual (média de 17,87%), com máximo teor no inverno, um dado convergente com Shanmugam & Seth (2018), que demonstraram variações sazonais na qualidade e quantidade de carragenana co-produzida com bioestimulantes de *K. alvarezii*. A carragenana é um polissacarídeo de alto valor comercial utilizado nas indústrias alimentícia, farmacêutica e de cosméticos (Rupert et al., 2022; Rudke, Andrade & Ferreira, 2020) e sua presença residual em teores superiores a 17% representa uma oportunidade concreta de valorização econômica do subproduto.

Além disso, a presença de carotenoides em concentrações de até 248,96 mg/100g no inverno, comparáveis a algumas fontes vegetais descritas na literatura (Chen, 2015), de flavonoides (13,54 mg/100g - outono) e de um perfil singular de ácidos graxos de cadeia longa (ácido heptadecanoico C17:0, majoritário no inverno; ácido nervônico C24:1n9 elevado em Florianópolis; ácido lignocérico C24:0 exclusivo em Palhoça) confere ao resíduo um potencial nutracêutico considerável, explorado de forma inédita nesta tese. As assinaturas sítio-específicas de ácidos graxos identificadas, um fenômeno que pode estar associado às diferenças nas correntes oceânicas e na temperatura local entre a Enseada do Brito (Palhoça) e o Ribeirão da Ilha (Florianópolis) (Piola & Matano, 2019), abrem uma perspectiva particularmente interessante para a rastreabilidade geográfica de produtos derivados da alga, um atributo de crescente valor em cadeias de qualidade certificada. Em conjunto, esses dados fornecem a base científica para um modelo de biorrefinaria em cascata sazonalmente orientado que, ao invés de tratar o resíduo como passivo ambiental, o transforma em múltiplos coprodutos de alto valor agregado, alinhado ao conceito de economia circular azul (Rudke, Andrade & Ferreira, 2020).

6.3. A hormese como princípio organizador da bioatividade

Os Capítulos 3 e 4 constituem o núcleo agronômico desta tese, e seus resultados revelam que o extrato aquoso de *K. alvarezii* atua não como um simples insumo nutritivo, o que sua composição em macronutrientes não sustentaria, dado o teor de nitrogênio abaixo do limite de detecção, mas como um modulador fisiológico cuja eficácia depende de forma crítica da dose e do estádio de desenvolvimento da planta. A janela de bioeficácia identificada para o *priming* de sementes de cevada (2–5% v/v), modelada pela equação de Brain-Cousens (BC.4), é coerente com a literatura de *priming* com extratos algais em outras espécies. Dutta et al. (2019) identificaram um intervalo ótimo de concentrações de extrato aquoso de *K. alvarezii* entre 2 e 5% em sementes de *Capsicum frutescens*. Por sua vez, Möller & Smith (1999) já haviam demonstrado, em trabalho seminal, que o *priming* de sementes de cevada com suspensões algais reduzia a sensibilidade à umidade das cariopses e melhorava o vigor germinativo. A convergência dessas faixas ótimas em culturas taxonomicamente distantes sugere que o fenômeno hormético identificado reflete uma sensibilidade conservada das plantas a elicitores derivados de algas marinhas em concentrações específicas, e não uma resposta espécie-dependente.

O mecanismo subjacente à resposta hormética observada no *priming* provavelmente envolve múltiplas moléculas sinalizadoras presentes no extrato. Com base na caracterização bioquímica das amostras de outono no Capítulo 1 (conteúdo de fenólicos totais = 16,78 mg/100g, aminoácidos livres = 3,23 mg/g e potássio como macronutriente dominante), e nos achados de Gandhi et al. (2024), que identificaram 198 compostos bioativos em extratos de *K. alvarezii* incluindo genisteína, ácido elágico e poliaminas, é possível inferir que os oligossacarídeos de κ-carragenana, liberados durante o processamento mecânico da biomassa, sejam os principais elicitores da atividade observada. Essa hipótese encontra suporte direto no trabalho de Rayorath et al. (2008), que demonstraram que extratos de *Ascophyllum nodosum* induzem atividade de α-amilase em sementes de cevada independentemente do ácido giberélico (GA_3), sugerindo que os bioativos algais podem ativar diretamente a cascata aleurônica GA→DELLA→α-amilase ou contorná-la por vias alternativas. A drástica queda monotônica no teor de amido total (59% entre o controle, concentração de 10% do extrato aquoso) e a concomitante inversão da razão amido: proteína (de 6,31 ± 0,28 para 1,95 ± 0,50) detectadas nesta tese são consistentes com esse mecanismo. Em seu conjunto, tais observações sugerem uma ativação do catabolismo do amido endospérmico durante a janela de embebição, mobilizando reservas de carboidratos para sustentar o anabolismo proteico e o arranque metabólico das plântulas. Essa reprogramação metabólica precoce, evidenciada pelo heatmap Z-score de variáveis bioquímicas, representa um mecanismo de preparação fisiológica (i.e., *priming stricto sensu*) que antecede e potencializa o desenvolvimento pós-germinativo.

No que se refere ao incremento de cerca de 20% nas clorofilas e carotenoides nas plântulas tratadas com o extrato aquoso a 2% v/v, pode-se recorrer ao papel duplo dos carotenoides. Estes são pigmentos acessórios na captura de luz e *quenchers* de ROS em condições de estresse (Chen, 2015; Nguyễn & Sung, 2025). Assim, sugere-se que o extrato, em concentrações intermediárias, estimulou o desenvolvimento cloroplastídico e potencializou a eficiência fotossintética inicial das plântulas.

6.4. Qualidade de grãos para a cadeia cervejeira

O resultado de maior impacto agronômico desta tese é a melhoria da qualidade bioquímica dos grãos de cevada obtida com a aplicação foliar do extrato (Capítulo 4), sem qualquer prejuízo à arquitetura vegetativa das plantas ou à viabilidade germinativa dos grãos

colhidos. Os aumentos de até 19,3% no conteúdo de amido total (5%, v/v), 10,3% nas proteínas (10%, v/v) e, especialmente, 56,6% nos aminoácidos totais livres (1 e 2%, v/v), com germinação mantida acima de 98,5% em todos os tratamentos, representam um conjunto de melhorias diretamente alinhado às exigências qualitativas da indústria malteira. A demanda por nitrogênio livre (FAN — Free Amino Nitrogen), derivado da hidrólise de proteínas durante a maltagem, é um dos parâmetros mais críticos à fermentação cervejeira, influenciando diretamente a nutrição das leveduras, a formação de compostos de aroma e a estabilidade coloidal do produto final (Lekkas et al., 2005; Schepper & Courtin, 2024). O aumento nos aminoácidos totais observado no Capítulo 4 sugere, portanto, um potencial de incremento direto no FAN dos grãos colhidos, um atributo de alto valor comercial que pode ser explorado pela cadeia produtiva da cevada nacional, que já enfrenta desafio de suprir a demanda interna crescente (CONAB, 2025).

Resultados análogos, embora com diferentes espécies de algas e culturas, foram documentados por Cozzolino et al. (2021), que observaram incremento de 17% na produtividade e de 17,9% na eficiência do uso de nitrogênio em cevada cervejeira tratada com extrato de *Ecklonia máxima*. Adicionalmente, Goñi et al. (2021) demonstraram redução de 25% na adubação nitrogenada sem perda de rendimento em cevada tratada com extrato de *Ascophyllum nodosum*. O presente trabalho é, contudo, o primeiro a documentar melhorias com extrato de *K. alvarezii* em cevada, e, mais importante, a fazê-lo com material produzido no Sul do Brasil e com total domínio da tecnologia, eliminando a necessidade de importação e reduzindo a pegada de carbono associada ao transporte daquele bioproduto. Por fim, o fato de o extrato não alterar a arquitetura vegetativa (altura, afilhos, espigas, grãos por espiga), mas atuar seletivamente no enchimento e na qualidade bioquímica dos grãos, sugere que seu modo de ação nessa fase é predominantemente sinalizador — modulando vias de alocação de fotoassimilados para o grão — e não nutritivo, em coerência com a ausência de nitrogênio detectável no extrato.

6.5. Integração dos resultados e perspectiva sistêmica

O extrato aquoso de *K. alvarezii* cultivada em Santa Catarina pode ser considerado um bioinsumo de composição previsível e sazonalmente estruturado, cuja atividade biológica, seja ela hormética no *priming* e seletivamente qualitativa na aplicação foliar, é intrinsecamente

dependente da qualidade da matéria-prima. A temperatura da superfície do mar, como preditor ambiental da composição do extrato (Capítulo 1), oferece aos produtores um parâmetro de fácil monitoramento para otimizar o calendário de colheita: as estações frias devem ser priorizadas para a produção de bioestimulantes de alta potência, enquanto o verão, que favorece o acúmulo de biomassa, pode ser direcionado à produção de carragenana. O resíduo sólido, por sua vez, abre uma segunda frente econômica que transforma o subproduto da cadeia em ativo biotecnológico, carragenana residual, carotenoides, flavonoides e ácidos graxos funcionais, viabilizando um modelo de biorrefinaria que agrega valor a cada etapa do processamento e minimiza o impacto ambiental da produção (Rudke, Andrade & Ferreira, 2020).

Do ponto de vista da bioeconomia do oceano, esta tese estabelece uma ligação entre a aquicultura marinha catarinense e a intensificação ecológica da agricultura, dois setores que historicamente operam de forma independente. A validação técnica aqui apresentada, como curvas de dose-resposta, janelas de bioeficácia, dados de composição sazonal e efeitos mensuráveis sobre qualidade de grãos, fornece os subsídios necessários ao registro formal do produto junto aos órgãos competentes (MAPA), e contribui para que o Brasil possa desenvolver uma cadeia produtiva de bioestimulantes algais baseada em matéria-prima nacional e com total domínio tecnológico, reduzindo a dependência de importações de extratos de *Ascophyllum nodosum* que atualmente dominam o mercado (Brasil, 2025). Dessa forma, os resultados desta tese não apenas respondem às questões científicas propostas, mas se inserem em um contexto mais amplo de consolidação da biotecnologia marinha brasileira como ferramenta estratégica à soberania agroalimentar e à transição para sistemas de produção de baixo carbono (Rabhi et al., 2025; Shukla et al., 2025).

.

7. Conclusões

Os resultados obtidos ao longo dos quatro capítulos desta tese permitem estabelecer as seguintes conclusões:

A composição bioquímica e mineral dos extratos aquosos comerciais de *K. alvarezii* cultivada em Santa Catarina é significativamente influenciada pela sazonalidade, com as estações frias (outono e inverno) favorecendo o acúmulo de compostos bioativos (aminoácidos, carboidratos, fenóis) e de potássio, enquanto a temperatura da superfície do mar mostrou correlação negativa com a maioria desses metabólitos. A variabilidade entre os locais de cultivo (Palhoça e Florianópolis) foi secundária em relação à variação sazonal, indicando homogeneidade nos processos produtivos dos fornecedores avaliados.

O resíduo sólido proveniente da produção do extrato aquoso de *K. alvarezii* constitui uma matriz rica em cinzas, fibras, carragenana residual, carotenoides, flavonoides e ácidos graxos de interesse nutracêutico, com perfis modulados pela sazonalidade e pelo local de cultivo. Esses achados fundamentam um modelo de biorrefinaria em cascata sazonalmente orientado, que transforma o subproduto em coprodutos de alto valor agregado (carragenana, pigmentos, compostos fenólicos), alinhando-se aos princípios da economia circular azul.

O priming de sementes de cevada (*H. vulgare* cv. Rubi) com o extrato aquoso de *K. alvarezii* elicita uma resposta hormética característica, com janela ótima de bioeficácia entre 2 e 5% (v/v) na qual se observaram incrementos significativos no crescimento da parte aérea e radicular, no índice de vigor e nos teores de pigmentos fotossintéticos. O extrato induziu uma reprogramação metabólica precoce, evidenciada pela queda monotônica do amido total e pela redução acentuada da razão amido:proteína, refletindo uma transição do metabolismo de armazenamento de carbono para o anabolismo proteico. Esse perfil metabólico, potencialmente mediado pela ativação de amilases aleurônicas por componentes do extrato, prepara as plântulas para um arranque vigoroso.

A aplicação foliar do extrato em plantas de cevada cultivadas em vasos não alterou a arquitetura vegetativa mas melhorou seletivamente a qualidade bioquímica dos grãos, com aumentos nos teores de amido, proteínas e aminoácidos totais, sem prejuízo à germinação das sementes produzidas. Esse padrão de resposta, associado à ausência de nitrogênio detectável no extrato, indica um modo de ação predominantemente modulador da alocação de

fotoassimilados para os grãos, posicionando o produto como ferramenta promissora para agregação de valor à cadeia da cevada malteira nacional.

Os resultados obtidos nos quatro eixos de investigação demonstram, de forma articulada, que a macroalga *K. alvarezii* cultivada no Sul do Brasil constitui uma fonte promissora de produtos naturais de grande interesse biotecnológico, evidenciado pela atividade bioestimulante hormética de seu extrato aquoso validada em cevada e pelo potencial de valorização integral de sua biomassa (incluindo o resíduo sólido). A abordagem multidisciplinar empregada, abrangendo desde a caracterização bioquímica sazonal e a análise de coprodutos até ensaios de dose-resposta e modelagem hormética, reforça o papel central da biotecnologia no desenvolvimento de insumos de base biológica. Essas evidências posicionam os produtos derivados da alga como ferramentas tecnológicas confiáveis para a intensificação ecológica da agricultura nacional, contribuindo para a consolidação de uma cadeia produtiva de bioestimulantes algais de base nacional sob o viés da biotecnologia marinha.

8. Perspectivas para trabalhos futuros

8.1.Validação agronômica em escala de campo.

Os experimentos desta tese foram conduzidos em condições controladas. A próxima etapa indispensável é a validação dos efeitos bioestimulantes do extrato de *K. alvarezii* em cevada sob condições reais de cultivo, com diferentes regimes de adubação nitrogenada e em situações de estresse abiótico (déficit hídrico, temperaturas supraótimas), seguindo como referência os delineamentos de campo plurianuais de Cozzolino et al. (2021) e Goñi et al. (2021).

8.2. Elucidação dos mecanismos moleculares de ação.

Os dados indicam que o extrato modula o catabolismo de amido e o anabolismo proteico no priming e sinaliza a alocação de fotoassimilados para o grão na aplicação foliar, mas os mecanismos subjacentes permanecem não elucidados. Abordagens transcriptômicas (Kumar et al., 2020a), proteômicas e de perfilamento de fitormônios, combinadas com ensaios de atividade enzimática (α-amilase, proteases, enzimas antioxidantes), poderiam testar a hipótese de ativação GA-independente da α-amilase (Rayorath et al., 2008) e vincular compostos específicos à resposta hormética.

8.3. Identificação metabolômica dos extratos.

O perfil hormético observado é consistente com a atividade de elicitores específicos, possivelmente oligossacarídeos de κ-carragenana ou fitormônios. Uma abordagem de metabolômica associada a LC-MS/MS permitiria identificar as frações responsáveis por cada aspecto da resposta, como crescimento radicular, catabolismo de amido, acúmulo de aminoácidos, abrindo caminho para a padronização de bioestimulantes com base em marcadores moleculares.

8.4. Investigação dos efeitos sobre a microbiota do solo e interações planta-micróbio.

Extratos de *K. alvarezii* modulam a microbiota rizosférica, enriquecendo filos associados ao metabolismo de N e S (Trivedi et al., 2021b; Nivetha et al., 2024). Esta vertente permanece inexplorada para a cevada. A avaliação por metagenômica dos efeitos do extrato e do resíduo sobre microrganismos benéficos do solo poderia revelar sinergias indiretas relevantes para o aumento da produtividade de plantas e maior resiliência a estresses bióticos e abióticos e (Pandey & Saharan, 2025; Yasmeen et al., 2025).

8.5. Análise tecno-econômica e de ciclo de vida do modelo de biorrefinaria.

O modelo de biorrefinaria em cascata proposto no Capítulo 2 requer uma análise de viabilidade financeira e ambiental. Uma análise de ciclo de vida (ACV) combinada a uma análise tecno-econômica (TEA) quantificaria as vantagens do modelo integrado sobre o descarte do resíduo, considerando os custos de processamento e os preços de mercado dos coprodutos.

8.6. Avaliação da estabilidade e vida de prateleira do extrato.

Um requisito fundamental para o registro comercial de um biostimulante e sua adoção em larga escala pelo setor agrícola é a garantia de estabilidade das propriedades bioativas ao longo do armazenamento. O extrato aquoso de *K. alvarezii* é um produto rico em compostos lábeis como fenóis, aminoácidos e pigmentos, e seu comportamento sob diferentes condições de temperatura, exposição à luz, atmosfera e formulação (líquido, concentrado, liofilizado) precisa ser caracterizado. Estudos de aceleração de envelhecimento, combinados com medidas de atividade biológica residual (como o priming de sementes de cevada utilizado nesta tese como bioensaio padronizado), forneceriam as informações necessárias para estabelecer diretrizes de acondicionamento e prazo de validade, requisitos indispensáveis tanto para o processo de registro no MAPA quanto para a confiança do agricultor no produto (Rabhi et al., 2025; Shukla et al., 2025).

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Anexo 1 - Material Suplementar do Capítulo 1

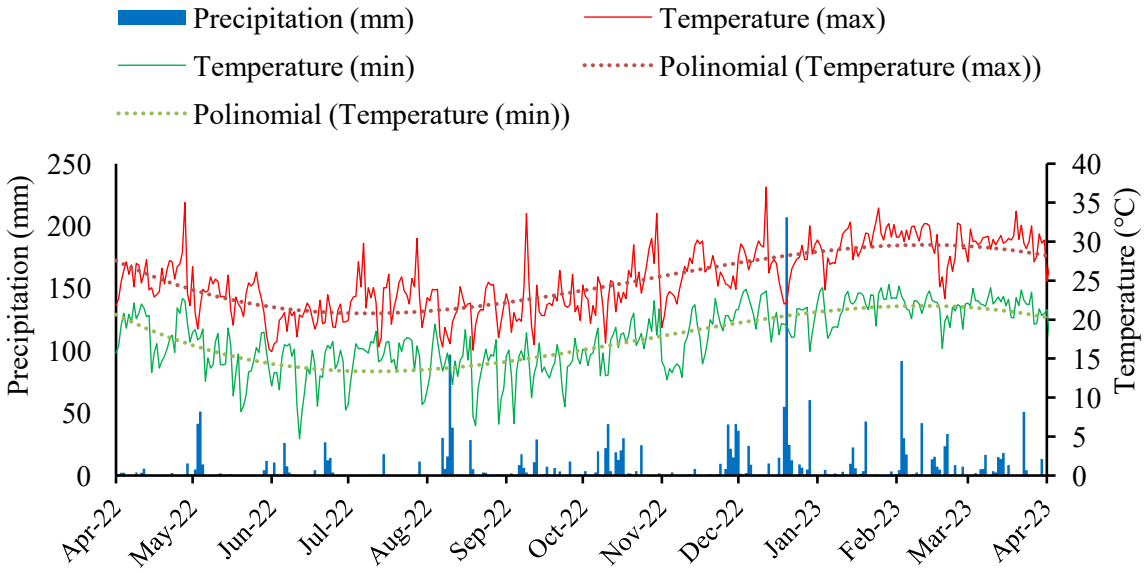

Fig. S1. Daily precipitation (mm) and maximum and minimum atmospheric temperature (°C) in Florianópolis region, southern Brazil, from April 2022 to April 2023. Source: INMET (2024).

Fig. S2. Score plot and factor loadings of principal component analysis (PCA) of biostimulant samples of *Kappaphycus alvarezii* cultivated in Palhoça (PAL) and Florianópolis (RIB), Southern Brazil, for total carbohydrates (TC), total soluble sugars (TSS), total starch (TS), total phenolic content (TPC), total protein (TP), and total amino acids (TAA). **A**: PC-1 and PC-2, **B**: PC-1 and PC-3.

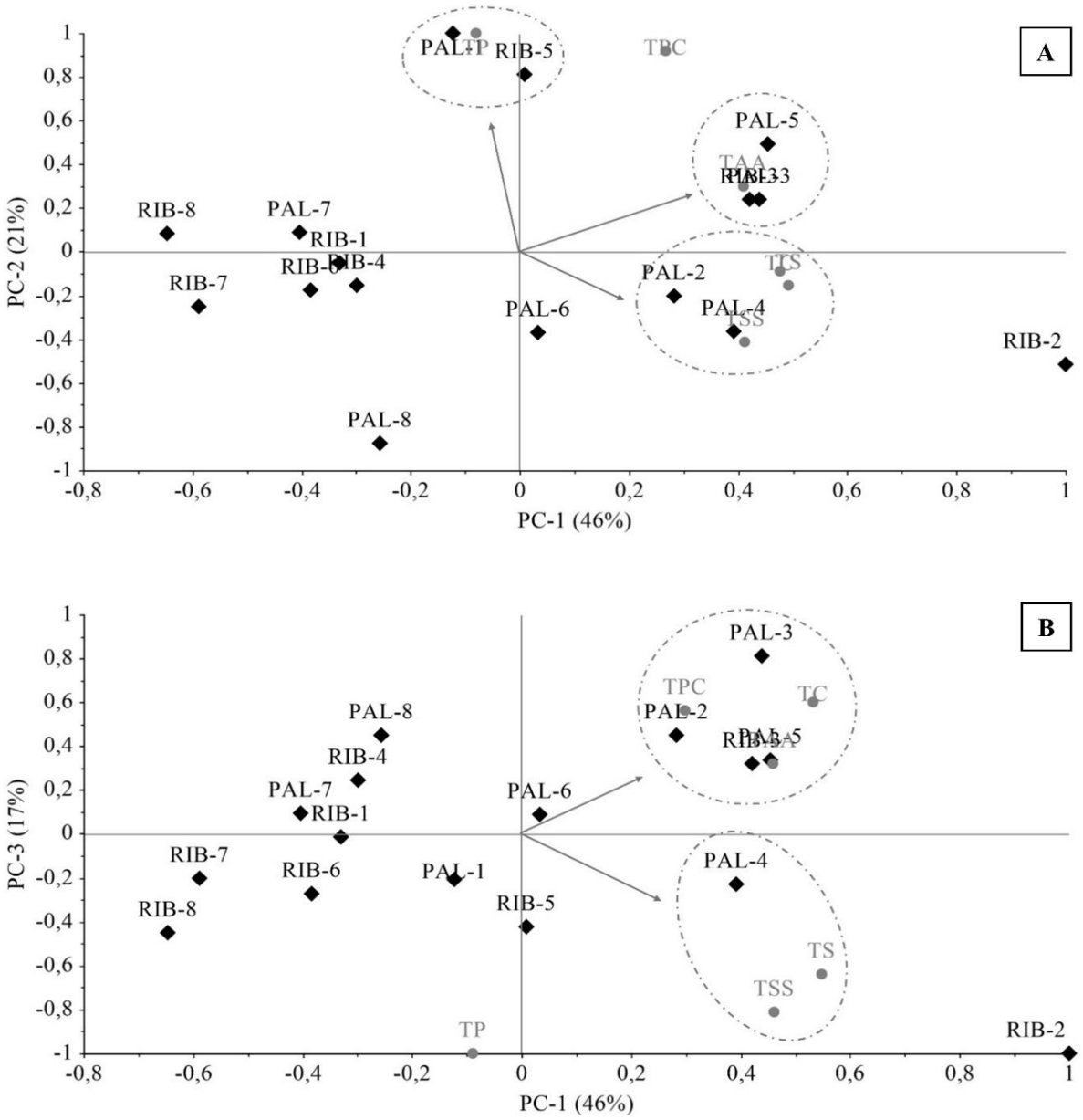

Fig. S3. Score plot and factor loadings of principal component analysis (PCA) of biostimulant samples of *Kappaphycus alvarezii* cultivated in Palhoça (PAL) and Florianópolis (RIB), Southern Brazil, for macro- and micronutrients. **A**: PC-1 and PC-2, **B**: PC-1 and PC-3.

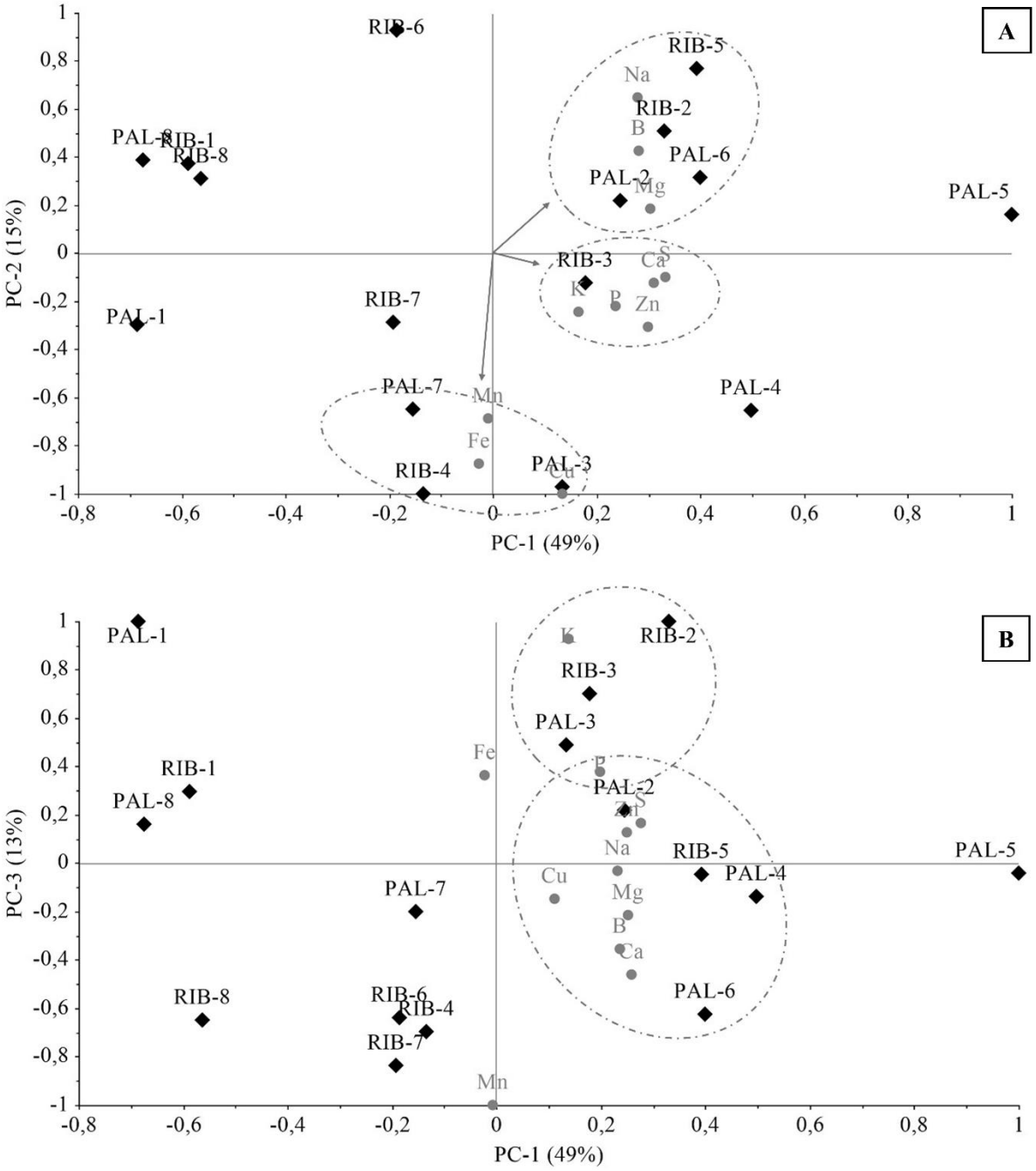

Table S1. Total solid content (TSC - %), pH, and conductivity (mS/cm) of biostimulants from *Kappaphycus alvarezii* cultivated in Palhoça (PAL) and Florianópolis (RIB), Southern Brazil, through the seasons.

Variable	Local	Sample	n	Autumn	Winter	Spring	Summer
TSC (%)	PAL	1	12	3.71 ± 0.20 ^{e}	4.03 ± 0.04 ^{d}	5.14 ± 0.03 ^{b}	4.22 ± 0.08 ^{d}
		2	12	3.99 ± 0.02 ^{d}	4.58 ± 0.03 ^{c}	4.13 ± 0.14 ^{d}	3.75 ± 0.54 ^{e}
	RIB	1	12	2.59 ± 0.05 ^{g}	4.60 ± 0.03 ^{c}	4.29 ± 0.16 ^{d}	4.14 ± 0.00 ^{d}
		2	12	5.55 ± 0.08 ^{a}	3.26 ± 0.02 ^{f}	3.65 ± 0.22 ^{e}	3.39 ± 0.08 ^{f}
	Average			***3.96 ± 1.11*** ^{A}	***4.12 ± 0.57*** ^{A}	***4.51 ± 1.11*** ^{A}	***4.20 ± 1.28*** ^{A}
pH	PAL	1	12	6.57 ± 0.01 ^{f}	6.56 ± 0.01 ^{f}	6.51 ± 0.06 ^{g}	5.01 ± 0.02 ^{m}
		2	12	6.56 ± 0.02 ^{f}	6.40 ± 0.01 ^{h}	6.73 ± 0.03 ^{d}	6.77 ± 0.01 ^{c}
	RIB	1	12	5.60 ± 0.02 ^{i}	5.23 ± 0.01 ^{l}	6.70 ± 0.01 ^{e}	5.58 ± 0.01 ^{j}
		2	12	6.51 ± 0.03 ^{g}	7.35 ± 0.05 ^{a}	7.00 ± 0.01 ^{b}	5.32 ± 0.01 ^{k}
	Average			***6.31 ± 0.43*** ^{A}	***6.38 ± 0.79*** ^{A}	***6.74 ± 0.19*** ^{A}	***5.67 ± 0.69*** ^{B}
Cond. (mS/cm)	PAL	1	12	46.12 ± 0.20 ^{n}	63.70 ± 0.02 ^{e}	73.07 ± 0.55 ^{c}	55.57 ± 0.15 ^{h}
		2	12	75.01 ± 0.61 ^{b}	64.97 ± 0.15 ^{d}	56.97 ± 0.23 ^{g}	53.77 ± 0.58 ^{k}
	RIB	1	12	44.18 ± 1.41 ^{o}	20.63 ± 0.49 ^{a}	53.10 ± 0.36 ^{l}	55.43 ± 0.20 ^{i}
		2	12	61.57 ± 0.12 ^{f}	53.80 ± 0.02 ^{j}	49.01 ± 0.63 ^{m}	39.76 ± 0.09 ^{p}
	Average			***56.72 ± 13.10*** ^{A}	***50.78 ± 18.73*** ^{A}	***58.04 ± 9.54*** ^{A}	***51.13 ± 6.90*** ^{A}

Means followed by the same letter do not differ statistically by the Scott-Knott test ($p \leq 0.05$), and Tukey test ($p \leq 0.05$) for average values.

Table S2. Total carbohydrates (TC - mg/g), total soluble sugars (TSS – mg/g), and total starch (TS - mg/g) of biostimulants from *Kappaphycus alvarezii* cultivated in Palhoça (PAL) and Florianópolis (RIB), Southern Brazil, through the seasons.

Variable	Local	Sample	n	Autumn	Winter	Spring	Summer
TC (mg/g)	PAL	1	12	3.36 ± 0.00 ^{h}	7.25 ± 0.02 ^{b}	8.48 ± 0.00 ^{a}	2.56 ± 0.00 ^{i}
		2	12	6.56 ± 0.00 ^{c}	4.96 ± 0.00 ^{f}	6.72 ± 0.00 ^{c}	6.19 ± 0.33 ^{d}
	RIB	1	12	2.56 ± 0.00 ^{i}	5.71 ± 0.02 ^{e}	4.25 ± 0.16 ^{g}	0.59 ± 0.09 ^{l}
		2	12	7.20 ± 0.00 ^{b}	2.03 ± 0.02 ^{j}	1.65 ± 0.09 ^{k}	0.16 ± 0.00 ^{m}
	Average			***4.92 ± 2.08*** ^{A}	***4.99 ± 1.98*** ^{A}	***5.28 ± 2.69*** ^{A}	***2.37 ± 2.49*** ^{B}
TSS (mg/g)	PAL	1	12	1.10 ± 0.09 ^{f}	2.82 ± 0.13 ^{d}	3.49 ± 0.21 ^{c}	1.07 ± 0.05 ^{f}
		2	12	2.49 ± 0.54 ^{d}	14.18 ± 0.43 ^{b}	3.91 ± 0.16 ^{c}	0.84 ± 0.05 ^{f}
	RIB	1	12	0.33 ± 0.10 ^{g}	1.27 ± 0.10 ^{f}	2.09 ± 0.08 ^{e}	0.47 ± 0.07 ^{g}
		2	12	17.39 ± 0.36 ^{a}	1.02 ± 0.41 ^{f}	0.61 ± 0.31 ^{g}	0.34 ± 0.07 ^{g}
	Average			***5.33 ± 7.33*** ^{A}	***4.82 ± 5.69*** ^{A}	***2.53 ± 1.37*** ^{B}	***0.68 ± 1.68*** ^{C}
TS (mg/g)	PAL	1	12	4.37 ± 0.03 ^{e}	3.85 ± 0.36 ^{f}	7.97 ± 0.33 ^{c}	1.08 ± 0.06 ^{j}
		2	12	6.65 ± 0.43 ^{d}	6.60 ± 0.13 ^{d}	6.18 ± 0.48 ^{d}	2.42 ± 0.18 ^{g}
	RIB	1	12	2.05 ± 0.26 ^{h}	9.73 ± 0.08 ^{b}	4.72 ± 0.33 ^{e}	2.05 ± 0.09 ^{h}
		2	12	18.37 ± 0.30 ^{a}	2.53 ± 0.26 ^{g}	4.00 ± 0.23 ^{f}	1.67 ± 0.24 ^{i}
	Average			***7.86 ± 6.57*** ^{A}	***5.68 ± 2.89*** ^{A}	***5.72 ± 1.61*** ^{A}	***1.80 ± 0.53*** ^{B}

Means followed by the same letter do not differ statistically by the Scott-Knott test ($p \leq 0.05$), and Tukey test ($p \leq 0.05$) for average values.

Table S3. Total protein (TP – mg/100 g), total amino acids (TAA – mg/g), and total phenolic contents (TPC – mg/100 g) of biostimulant samples of *Kappaphycus alvarezii* cultivated in Palhoça (PAL) and Florianópolis (RIB), Southern Brazil, through the seasons.

Variable	Local	Sample	n	Autumn	Winter	Spring	Summer
TP (mg/100 g)	PAL	1	12	45.63 ± 3.90 [a]	14.38 ± 2.87 [d]	27.50 ± 4.33 [c]	24.38 ± 5.63 [c]
		2	12	13.13 ± 4.96 [d]	16.25 ± 2.86 [d]	18.13 ± 3.90 [d]	5.31 ± 1.43 [e]
	RIB	1	12	26.25 ± 3.25 [c]	20.63 ± 1.88 [c]	50.63 ± 11.41 [a]	23.44 ± 2.81 [c]
		2	12	26.88 ± 2.17 [c]	15.00 ± 1.88 [d]	27.50 ± 2.86 [c]	35.00 ± 2.86 [b]
	Average			***27.97 ± 12.50*** [A]	***16.57 ± 3.28*** [B]	***30.94 ± 13.72*** [A]	***22.03 ± 11.54*** [AB]
TAA (mg/g)	PAL	1	12	1.63 ± 0.01 [k]	6.90 ± 0.01 [a]	2.97 ± 0.01 [g]	0.66 ± 0.01 [m]
		2	12	4.84 ± 0.01 [d]	2.94 ± 0.01 [h]	0.64 ± 0.01 [n]	0.74 ± 0.01 [l]
	RIB	1	12	3.25 ± 0.01 [f]	5.97 ± 0.01 [b]	4.68 ± 0.01 [e]	0.63 ± 0.01 [n]
		2	12	4.94 ± 0.01 [c]	2.14 ± 0.01 [h]	2.49 ± 0.01 [i]	0.52 ± 0.01 [o]
	Average			***3.67 ± 1.41*** [AB]	***4.48 ± 2.08*** [A]	***2.69 ± 1.50*** [B]	***0.64 ± 1.64*** [C]
TPC (mg/100 g)	PAL	1	12	22.33 + 1.90 [a]	18.93 + 2.57 [b]	23.17 + 2.49 [a]	12.63 + 4.43 [d]
		2	12	11.23 + 1.05 [d]	13.93 + 1.55 [c]	9.17 + 0.85 [e]	1.17 + 0.15 [g]
	RIB	1	12	4.83 + 0.29 [f]	16.30 + 1.80 [c]	12.00 + 0.30 [d]	4.07 + 0.64 [f]
		2	12	6.90 + 0.27 [e]	11.00 + 0.70 [d]	2.23 + 1.72 [g]	4.97 + 1.37 [f]
	Average			***11.33 ± 7.13*** [A]	***15.04 ± 3.42*** [A]	***11.64 ± 7.99*** [A]	***5.71 ± 4.86*** [B]

Means followed by the same letter do not differ statistically by the Scott-Knott test ($p \leq 0.05$), and Tukey test ($p \leq 0.05$) for average values.

Anexo 2 - Material Suplementar do Capítulo 2

Table S1. Results of one-way statistical tests comparing the eight treatment groups (season × location combinations: PAL-Aut, PAL-Win, PAL-Spr, PAL-Sum, RIB-Aut, RIB-Win, RIB-Spr, RIB-Sum) for each biochemical variable of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production. Normality of residuals was assessed by the Shapiro–Wilk test and homoscedasticity by Levene's test; variables meeting both assumptions were analysed by one-way ANOVA, and the remaining variables by the Kruskal–Wallis test. Significance codes: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns = not significant.

Variable	Description	Statistical test	Statistic	p-value	Sig.
Proximate composition					
Moisture	Moisture content (%)	Kruskal–Wallis	H = 16.37	0.0220	*
Ash	Ash content (%)	Kruskal–Wallis	H = 37.81	< 0.001	***
TP	Total protein (mg g^{-1})	Kruskal–Wallis	H = 28.95	< 0.001	***
TL	Total lipids (%)	One-way ANOVA	F = 0.862	0.5442	ns
Carbohydrate fractions					
NDF	Neutral detergent fibre (%)	Kruskal–Wallis	H = 22.33	0.0022	**
TC	Total carbohydrates (mg g^{-1})	Kruskal–Wallis	H = 29.07	< 0.001	***
TSS	Total soluble sugars (mg g^{-1})	Kruskal–Wallis	H = 18.22	0.0110	*
TS	Total starch (mg g^{-1})	Kruskal–Wallis	H = 34.46	< 0.001	***
Bioactive compounds					
TCN	Total carotenoids (mg 100 g^{-1})	Kruskal–Wallis	H = 23.17	0.0016	**
TPC	Total phenolic compounds (mg 100 g^{-1})	Kruskal–Wallis	H = 35.45	< 0.001	***
TFC	Total flavonoid compounds (mg 100 g^{-1})	Kruskal–Wallis	H = 33.73	< 0.001	***
Carrageenan					
Yield	Carrageenan yield (%)	One-way ANOVA	F = 3.448	0.0031	**

Table S2. Principal Component scores of the biochemical composition of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production, cultivated in Palhoça (PAL) and Florianópolis (RIB), Santa Catarina, Brazil, sampled across four seasons over one year.

Sample	PC1	PC2	PC3
PAL-1	-1.348	0.352	1.419
PAL-2	-2.794	-1.091	0.121
PAL-3	0.080	-1.600	-0.148
PAL-4	0.006	-0.480	0.578
PAL-5	0.425	-1.592	0.678
PAL-6	2.473	-0.850	2.300
PAL-7	0.569	-0.480	0.340
PAL-8	-2.491	0.279	2.178
RIB-1	-0.485	-1.628	-1.724
RIB-2	-1.058	-0.732	-1.602
RIB-3	-0.199	-0.033	-1.698
RIB-4	-2.567	2.198	-0.509
RIB-5	0.903	4.076	0.555
RIB-6	2.098	0.121	0.534
RIB-7	0.563	1.269	-2.232
RIB-8	3.824	0.190	-0.790

Table S3. Principal Component loadings of the biochemical composition of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production, cultivated in Palhoça (PAL) and Florianópolis (RIB), Santa Catarina, Brazil, sampled across four seasons over one year.

Variable	PC1	PC2	PC3
Moisture	-0.178	0.422	0.170
Ashes	-0.302	-0.384	0.254
TP	-0.377	-0.005	-0.099
TL	0.014	0.326	0.451
NDF	0.155	-0.346	0.433
TC	-0.418	-0.331	-0.188
TSS	-0.365	0.003	0.202
TS	-0.266	0.042	-0.521
TCN	-0.160	0.536	0.041
TPC	-0.388	-0.110	0.332
TFC	-0.352	0.164	0.124
Carrageenan	0.179	-0.104	0.178

Table S4. Principal Component scores of the mineral composition of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production, cultivated in Palhoça (PAL) and Florianópolis (RIB), Santa Catarina, Brazil, sampled across four seasons over one year.

Sample	PC1	PC2	PC3
PAL-1	-2.509	-0.178	0.981
PAL-2	-1.608	-0.115	-0.867
PAL-3	-1.391	-0.558	-0.354
PAL-4	0.740	0.381	-2.973
PAL-5	0.774	-3.798	-0.356
PAL-6	-0.781	-0.953	0.658
PAL-7	1.464	-0.238	-0.759
PAL-8	-2.842	1.505	0.184
RIB-1	-0.842	1.685	0.863
RIB-2	-2.387	-0.566	-0.719
RIB-3	1.117	1.757	-1.019
RIB-4	1.816	1.326	-1.552
RIB-5	1.142	-1.821	0.458
RIB-6	1.988	-0.035	1.597
RIB-7	2.522	0.980	0.806
RIB-8	0.796	0.628	3.053

Table S5. Principal Component loadings of the mineral composition of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production, cultivated in Palhoça (PAL) and Florianópolis (RIB), Santa Catarina, Brazil, sampled across four seasons over one year.

Variável	PC1	PC2	PC3
N	-0.268	0.386	-0.241
P	-0.349	0.014	-0.434
K	0.450	-0.051	0.279
Ca	0.218	-0.386	-0.047
Mg	0.295	-0.314	-0.459
S	0.121	-0.436	-0.177
B	0.420	0.181	0.154
Cu	0.148	0.220	0.211
Fe	0.261	0.368	-0.301
Mn	0.351	0.440	-0.194
Zn	0.255	0.010	-0.485

Table S6. Principal Component scores of the fatty acids of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production, cultivated in Palhoça (PAL) and Florianópolis (RIB), Santa Catarina, Brazil, sampled across four seasons over one year.

Sample	PC1	PC2	PC3
PAL-1	0.081	-0.377	0.154
PAL-2	1.624	-1.022	-1.363
PAL-3	-0.385	0.116	-0.071
PAL-4	-5.160	-0.117	-1.167
PAL-5	-2.722	-0.000	-0.656
PAL-6	0.912	1.852	0.312
PAL-7	1.347	1.689	0.845
PAL-8	2.385	-0.874	1.678
RIB-1	-0.628	-0.945	-0.289
RIB-2	-0.156	1.234	-1.892
RIB-3	0.478	0.567	-0.412
RIB-4	-1.234	-0.678	0.567
RIB-5	-2.891	-1.456	0.789
RIB-6	-3.456	-0.234	1.123
RIB-7	-1.789	0.345	-0.567
RIB-8	-3.012	-1.012	-0.345

Table S7. Principal Component loadings of the fatty acids of the industrial solid residue of *Kappaphycus alvarezii* obtained after aqueous extract production, cultivated in Palhoça (PAL) and Florianópolis (RIB), Santa Catarina, Brazil, sampled across four seasons over one year.

Fatty Acids	PC1	PC2	PC3
C12:0	-0.463	-0.018	-0.297
C14:1	0.249	0.546	-0.034
C17:0	0.458	-0.263	-0.234
C17:1	0.179	-0.356	0.639
C18:2n6t	0.416	-0.029	-0.061
C18:2n6c	0.268	0.295	-0.450
C22:2	-0.021	0.525	0.443
C24:0	-0.487	0.028	-0.041
C24:1n9	-0.007	0.377	0.209

Anexo 3 - Material Suplementar do Capítulo 3

Table S1 – Mean ± standard deviation of all morphological, physiological, and biochemical variables measured in barley (*Hordeum vulgare* L. cv. Rubi) seedlings after seed priming with *Kappaphycus alvarezii* aqueous extract at seven concentrations (0–20% v/v). Different lowercase letters within a row denote statistically significant differences among concentrations at *p* < 0.05 (Tukey HSD post-hoc test following one-way ANOVA; Dunn–Bonferroni post-hoc test following Kruskal–Wallis rank-sum test, according to the statistical framework indicated in Table S2)

Variable	0%	0.5%	1%	2%	5%	10%	20%
Germination (%)	0.95 ± 0.04 a	0.91 ± 0.05 a	0.92 ± 0.02 a	0.93 ± 0.05 a	0.94 ± 0.00 a	0.86 ± 0.04 a	0.84 ± 0.06 a
Fresh shoot mass (mg)	110.96 ± 18.45 e	121.78 ± 13.08 cde	130.38 ± 20.73 bcd	142.05 ± 22.60 ab	149.88 ± 23.54 a	137.31 ± 17.99 abc	114.86 ± 15.15 de
Dry shoot mass (mg)	11.49 ± 2.36 bc	12.89 ± 2.41 ab	12.53 ± 1.83 ab	13.08 ± 2.37 ab	13.70 ± 2.22 a	11.65 ± 1.78 bc	10.20 ± 1.51 c
Fresh root mass (mg)	50.41 ± 10.22 b	71.21 ± 11.63 a	77.91 ± 17.11 a	80.07 ± 15.35 a	81.97 ± 13.79 a	79.31 ± 14.96 a	71.06 ± 9.65 a
Dry root mass (mg)	5.67 ± 1.29 c	7.69 ± 1.43 ab	7.70 ± 1.90 ab	7.04 ± 1.72 b	8.54 ± 1.80 a	7.27 ± 1.51 b	6.79 ± 1.03 bc
Root:Shoot ratio	0.46 ± 0.09 b	0.59 ± 0.09 a	0.60 ± 0.12 a	0.57 ± 0.13 a	0.56 ± 0.11 a	0.58 ± 0.11 a	0.62 ± 0.07 a
Shoot length (cm)	11.30 ± 1.34 c	12.26 ± 1.89 b	13.61 ± 1.56 a	13.56 ± 1.53 a	13.56 ± 1.71 a	12.58 ± 2.04 b	9.49 ± 3.02 d
Root length (cm)	6.02 ± 1.80 d	8.36 ± 2.15 b	9.39 ± 2.11 a	9.28 ± 2.32 a	9.47 ± 2.19 a	9.25 ± 1.93 a	7.38 ± 2.09 c
Seedling Vigor Index (SVI)	10.79 ± 1.35 b	11.14 ± 1.85 b	12.59 ± 1.45 a	12.65 ± 1.54 a	12.74 ± 1.60 a	10.82 ± 1.81 b	7.95 ± 2.62 c
Chlorophyll a (mg g-1)	0.63 ± 0.02 d	0.69 ± 0.00 bc	0.69 ± 0.01 bc	0.76 ± 0.03 a	0.65 ± 0.02 cd	0.70 ± 0.01 b	0.71 ± 0.02 ab
Chlorophyll b (mg g-1)	0.22 ± 0.01 d	0.24 ± 0.00 bc	0.24 ± 0.00 bc	0.26 ± 0.01 a	0.23 ± 0.00 cd	0.24 ± 0.00 bc	0.24 ± 0.01 b
Total chlorophyll (mg g-1)	0.85 ± 0.02 d	0.93 ± 0.00 bc	0.92 ± 0.01 bc	1.02 ± 0.04 a	0.88 ± 0.02 cd	0.94 ± 0.01 bc	0.95 ± 0.03 b
Carotenoids (mg g-1)	0.12 ± 0.00 b	0.14 ± 0.00 ab	0.13 ± 0.01 ab	0.14 ± 0.01 a	0.13 ± 0.00 ab	0.14 ± 0.00 ab	0.14 ± 0.01 ab
Total soluble sugars (TSS) (mg g-1)	37.36 ± 1.90 a	42.67 ± 1.16 a	57.27 ± 12.19 a	51.42 ± 7.67 a	45.04 ± 9.17 a	39.80 ± 2.18 a	55.13 ± 1.38 a
Total starch (TS) (mg g-1)	10.25 ± 0.65 a	8.47 ± 0.86 ab	9.08 ± 0.85 ab	6.30 ± 0.08 cd	8.04 ± 0.08 bc	4.21 ± 1.06 e	4.85 ± 0.42 de
Total protein (TP) (mg g-1)	1.62 ± 0.08 ab	1.47 ± 0.07 b	1.47 ± 0.05 ab	2.01 ± 0.18 ab	2.21 ± 0.05 a	2.16 ± 0.02 ab	2.09 ± 0.30 ab
Starch:Protein ratio	6.31 ± 0.28 a	5.80 ± 0.88 a	6.18 ± 0.39 a	3.14 ± 0.30 bc	3.64 ± 0.06 b	1.95 ± 0.50 c	2.34 ± 0.27 c
Free amino acids (TAA) (mg g-1)	4.14 ± 0.16 ab	2.60 ± 0.04 b	4.24 ± 0.47 ab	4.87 ± 0.33 a	2.68 ± 0.25 b	4.74 ± 0.17 a	3.38 ± 0.63 ab

Table S2 – Summary of statistical analyses for the effect of *Kappaphycus alvarezii* aqueous extract concentration on each response variable. One-way ANOVA (*F* statistic) was used when assumptions of normality and homoscedasticity were met; the Kruskal–Wallis test (*H* statistic) was applied otherwise. Significance levels: * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001.

Variable	Test	Statistic	Value	p_value	Sig
Germination (%)	kw	H	144.836	0.0247	*
Fresh shoot mass (mg)	anova	F	114.182	0.0000	***
Dry shoot mass (mg)	anova	F	63.799	0.0000	***
Fresh root mass (mg)	kw	H	485.279	0.0000	***
Dry root mass (mg)	kw	H	341.709	0.0000	***
Root:Shoot ratio	kw	H	272.639	0.0001	***
Shoot length (cm)	kw	H	1.890.068	0.0000	***
Root length (cm)	kw	H	1.356.892	0.0000	***
Seedling Vigor Index (SVI)	kw	H	2.308.069	0.0000	***
Chlorophyll a (mg g-1)	anova	F	170.822	0.0000	***
Chlorophyll b (mg g-1)	anova	F	186.133	0.0000	***
Total chlorophyll (mg g-1)	anova	F	175.899	0.0000	***
Carotenoids (mg g-1)	kw	H	151.342	0.0192	*
Total soluble sugars (TSS) (mg g-1)	kw	H	147.152	0.0226	*
Total starch (TS) (mg g-1)	anova	F	331.868	0.0000	***
Total protein (TP) (mg g-1)	kw	H	169.839	0.0093	**
Starch:Protein ratio	anova	F	513.143	0.0000	***
Free amino acids (TAA) (mg g-1)	kw	H	171.887	0.0086	**

Table S3 – Principal component analysis (PCA) of morphophysiological variables measured in barley seedlings after seed priming with *Kappaphycus alvarezii* aqueous extract (0–20% v/v). (A) Variance explained by the first six principal components. (B) Contribution (%) of each morphophysiological variable to the first five dimensions. (C) Scores of each treatment concentration along the first five principal components.

(A) Variance explained	
Principal component	Variance (%)
PC1	68.43
PC2	24.10
PC3	4.46
PC4	1.94
PC5	0.62
PC6	0.44
(B) Variable contributions (%)	

Variable	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
Fresh shoot mass (mg)	18.18	0.23	13.94	57.65	2.61
Dry shoot mass (mg)	15.00	13.37	9.26	0.29	58.79
Fresh root mass (mg)	15.06	15.31	1.55	0.01	4.41
Dry root mass (mg)	15.35	4.03	60.17	0.06	18.88
Shoot length (cm)	16.44	8.05	11.12	26.13	5.72
Root length (cm)	18.43	5.06	3.83	14.57	0.01
Germination (%)	1.54	53.95	0.15	1.31	9.59
(C) Treatment scores					
Concentration (v/v)	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
0%	−3.224	2.031	−0.175	0.111	0.092
0.5%	0.065	0.204	0.812	−0.318	−0.281
1%	1.183	0.161	−0.007	−0.588	0.259
2%	1.474	0.506	−0.727	0.101	−0.283
5%	2.715	0.367	0.489	0.550	0.152
10%	0.466	−1.321	−0.608	−0.057	0.057
20%	−2.679	−1.948	0.217	0.202	0.004

Table S4 – Principal component analysis (PCA) of biochemical variables measured in barley seedling shoots after seed priming with *Kappaphycus alvarezii* aqueous extract (0–20% v/v). (A) Variance explained by the first six principal components. (B) Contribution (%) of each biochemical variable to the first five dimensions. (C) Scores of each treatment concentration along the first five principal components.

(A) Variance explained	
Principal component	Variance (%)
PC1	61.62
PC2	16.96
PC3	11.96
PC4	6.50
PC5	2.19
PC6	0.77

(B) Variable contributions (%)					
Variable	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
Chlorophyll a (mg g^{-1})	19.53	1.48	0.16	2.68	0.28
Chlorophyll b (mg g^{-1})	18.32	2.89	0.24	5.32	12.80
Total chlorophyll (mg g^{-1})	19.30	1.81	0.18	3.29	1.74
Carotenoids (mg g^{-1})	17.97	0.01	4.80	4.05	2.45
Total soluble sugars (TSS) (mg g^{-1})	6.10	18.88	13.05	61.09	0.44
Total starch (TS) (mg g^{-1})	11.82	23.37	0.27	0.20	51.07
Total protein (TP) (mg g^{-1})	3.47	51.52	1.76	11.21	30.94

Free amino acids (TAA) (mg g^{-1})	3.50	0.05	79.52	12.15	0.28
(C) Treatment scores					
Concentration (v/v)	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
0%	−3.517	0.005	1.180	0.062	0.166
0.5%	−0.654	0.854	−0.810	−1.369	−0.199
1%	−0.209	1.785	0.125	0.833	−0.278
2%	3.346	0.474	0.691	−0.212	0.608
5%	−1.501	−1.112	−1.158	0.296	0.471
10%	1.075	−1.616	0.950	−0.216	−0.491
20%	1.460	−0.389	−0.978	0.605	−0.277

Anexo 4 - Material Suplementar do Capitulo 4

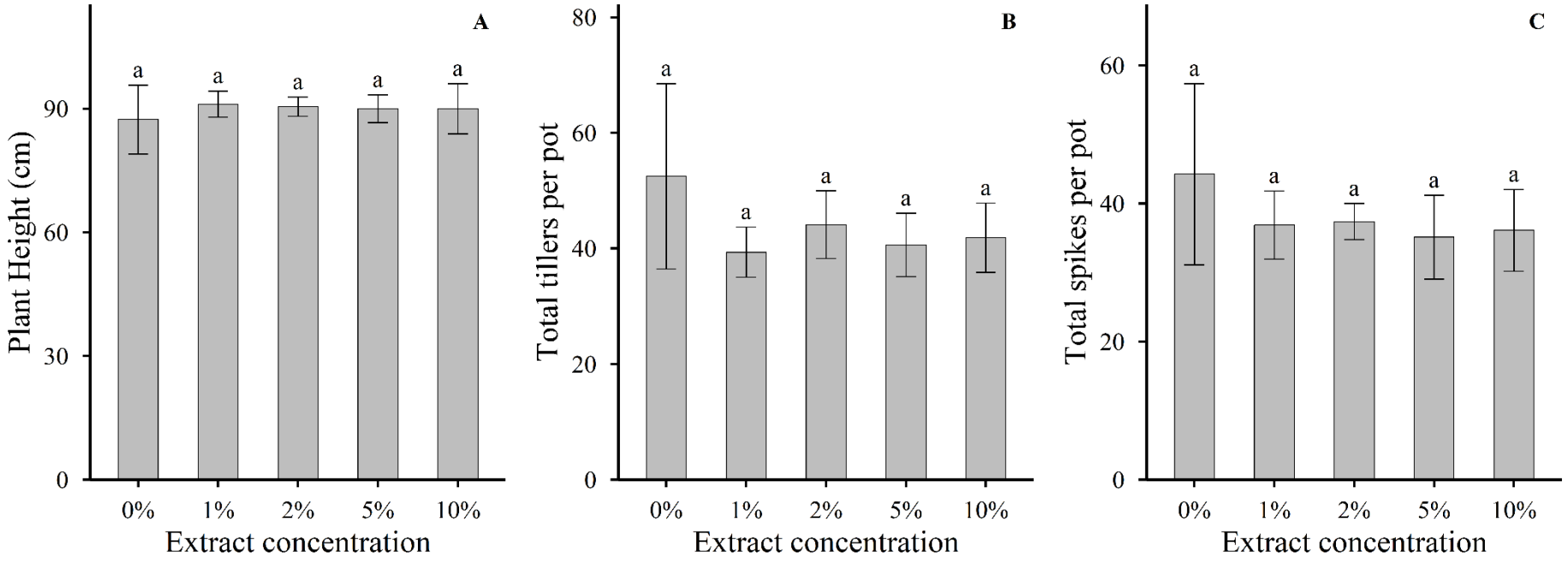

Fig. S1. Morphological parameters of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses diluted in water (Control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil. A - Plant height (PH – cm); B - total tillers per pot (TTP – n.), C - total spikes per pot (TSP – n.). Values followed by different letters differ significantly for the Dunn's test with Holm-Bonferroni adjustment ($p < 0.05$).

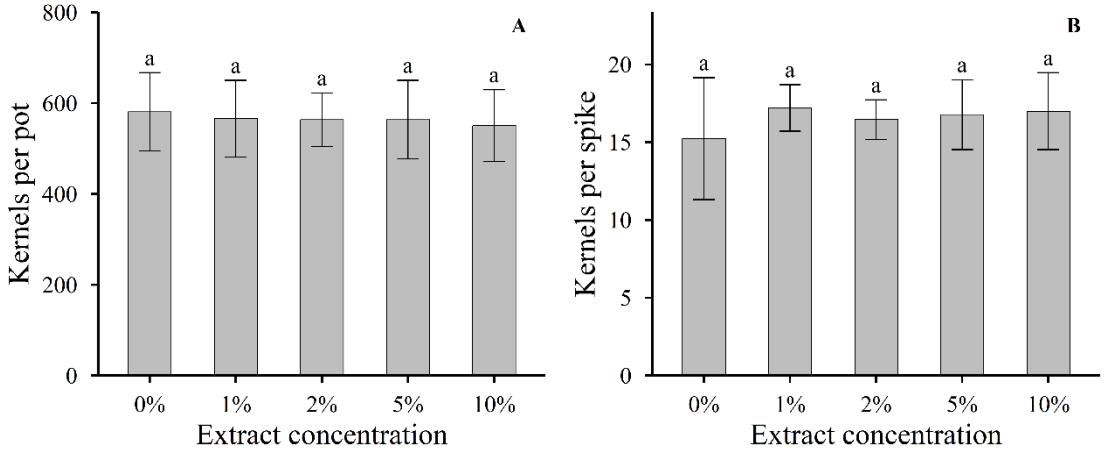

Fig. S2. Yield components of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses diluted in water (Control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil. A - Number of kernels per pot (NKP - n); B - number of kernels per spike (NKS – n.). Values followed by different letters differ significantly for the Dunn's test with Holm-Bonferroni adjustment ($p < 0.05$).

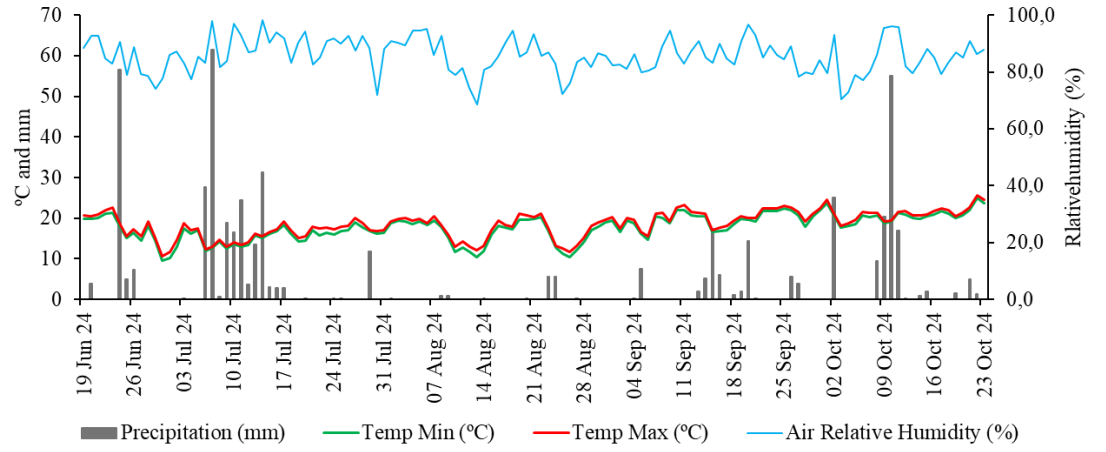

Fig. S3. Precipitation (mm), Temperatures min and max (ºC) and Air Relative Humidity (%) during the barley (*Hordeum vulgaris* L.) cultivation in the greenhouse on Epagri Training Center (CETRE) in Florianópolis, Santa Catarina, Brazil.