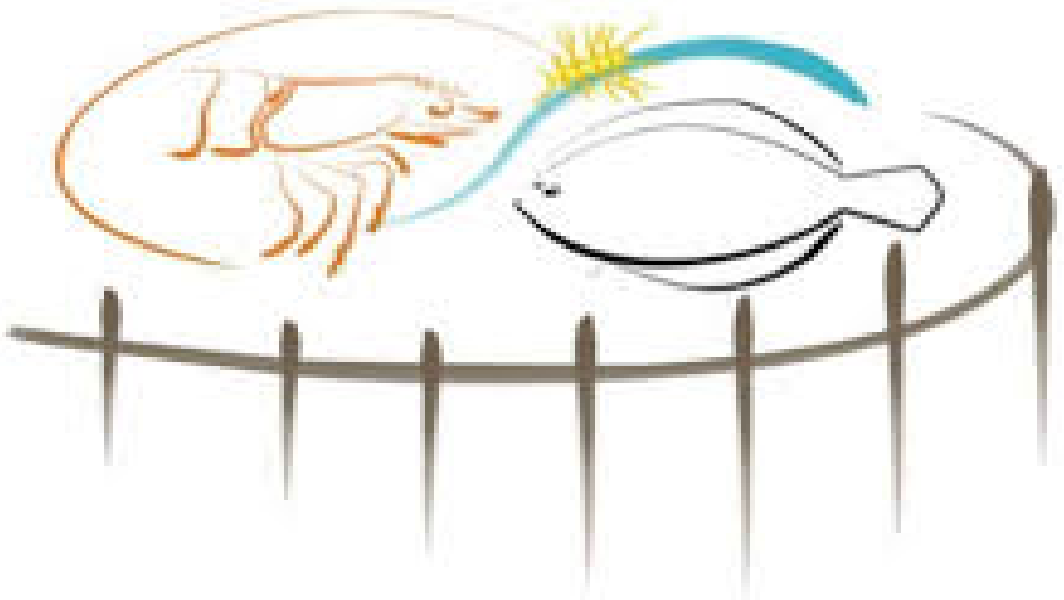
UNIVERSIDADE FEDERAL DO RIO GRANDE – FURG

INSTITUTO DE OCEANOGRAFIA – IO

PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA

PLANTAS HALÓFITAS NATIVAS DO BRASIL: COMPOSTOS FENÓLICOS, PIGMENTOS, ATIVIDADE ANTIOXIDANTE E USO NA ALIMENTAÇÃO DE CAMARÕES MARINHOS (*Litopenaeus vannamei* Boone, 1931)



MSc. MANUEL CEZAR MACEDO BARBOSA NOGUEIRA DE SOUZA

RIO GRANDE, RS

2018

UNIVERSIDADE FEDERAL DO RIO GRANDE - FURG
INSTITUTO DE OCEANOGRAFIA – IO
PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA

PLANTAS HALÓFITAS NATIVAS DO BRASIL: COMPOSTOS FENÓLICOS, PIGMENTOS, ATIVIDADE ANTIOXIDANTE E USO NA ALIMENTAÇÃO DE CAMARÕES MARINHOS (*Litopenaeus vannamei* Boone, 1931)

MSc. MANUEL CEZAR MACEDO BARBOSA NOGUEIRA DE SOUZA

Orientador: Prof. Dr. César Serra Bonifácio Costa
Co-orientadora: Profa. Dra. Eliana Badiale-Furlong

Tese apresentada como parte dos requisitos para obtenção do grau de **DOUTOR** em Aquicultura no Programa de Pós-Graduação em Aquicultura da Universidade Federal do Rio Grande.

RIO GRANDE, RS
MAIO / 2018

Ficha catalográfica

S729p Souza, Manuel Cezar Macedo Barbosa Nogueira de.
Plantas halófitas nativas do Brasil: compostos fenólicos, pigmentos, atividade antioxidante e uso na alimentação de camarões marinhos (*Litopenaeus vannamei Boone*, 1931) / Manuel Cezar Macedo Barbosa Nogueira de Souza. – 2018.
143 p.

Tese (doutorado) – Universidade Federal do Rio Grande – FURG, Programa de Pós-graduação Aquicultura, Rio Grande/RS, 2018.
Orientador: Dr. César Serra Bonifácio Costa.
Coorientadora: Drª. Eliana Badiale-Furlong.

1. Efeito da salinidade na composição da biomassa 2. Compostos bioativos 3. Efluente de aquicultura 4. Ração para *Litopenaeus vannamei* I. Costa, César Serra Bonifácio II. Badiale-Furlong, Eliana III. Título.

CDU 639.3

Catalogação na Fonte: Bibliotecário Me. João Paulo Borges da Silveira CRB 10/2130

Pós Graduação em
AQUICULTURA
Universidade Federal do Rio Grande

ATA 03/2018

DE DEFESA DA 51ª TESE DE DOUTORADO EM AQUICULTURA

No dia quatorze de maio de dois mil e dezoito, às quatorze horas, no Auditório da Estação Marinha de Aquacultura da FURG, reuniu-se a Banca Examinadora de Tese de Doutorado em Aquicultura, do **MANUEL CEZAR MACEDO BARBOSA DE SOUZA**, orientado pelo Professor. Dr. Cesar Serra Bonifácio Costa, co-orientado pela Profa. Dra. Eliana Badiale Furlong composta pelos seguintes membros: Prof. Dr. Cesar Serra Bonifácio Costa (Orientador – IO/FURG), Profa. Dra. Eliana Badiale Furlong (Co-orientadora – EQA/FURG), Prof. Dr. Marcelo Borges Tesser (IO/FURG), Profa. Dra. Larine Kupski (EQA/FURG) e a Profa. Dra. Cristina Copstein Cuchiara (IFRS). Título da Tese: **"PLANTAS HALÓFITAS NATIVAS DO BRASIL: COMPOSTOS FENÓLICOS, PIGMENTOS, ATIVIDADE ANTIOXIDANTE E USO NA ALIMENTAÇÃO DE CAMARÕES MARINHOS (*Litopenaeus vannamei* Boone, 1931)".** Dando início à defesa, o Coordenador e Orientador, Prof. Dr. Luis Henrique da Silva Poersch, passou a presidência da sessão ao Prof. Dr. Cesar Serra Bonifácio Costa, que na qualidade de orientador, passou a palavra para o candidato apresentar a Tese. Após ampla discussão entre os membros da Banca e o candidato, a Banca se reuniu sob a presidência do Coordenador. Durante esse encontro ficou estabelecido que as sugestões dos membros da Banca Examinadora devem ser incorporadas na versão final, ficando a cargo do Orientador o cumprimento desta decisão. O candidato **MANUEL CEZAR BARBOSA NOGUEIRA DE SOUZA** foi considerado **APROVADO**, devendo a versão definitiva da Tese ser entregue na Secretaria do PPGAq, no prazo estabelecido nas Normas Complementares do Programa. Nada mais havendo a tratar, foi lavrada a presente ata, que após lida e aprovada, será assinada pela Banca Examinadora, pelo candidato e pelo Coordenador do PPGAq.

PROF. DR. CESAR SERRA BONIFACIO COSTA ORIENTADOR (IO/FURG)

PROFA. DRA. ELIANA BADIALE FURLONF CO-ORIENTADORA (EQA/FURG)

PROF. DR. MARCELO BORGES TESSER (IO/FURG)

PROFA. DRA. LARINE KUPSKI (EQA/FURG)

PROFA. DRA. CRISTINA CUPSTEIN CUCHIARA (IFRS)

MANUEL CEZAR NOGUEIRA BARBOSA DE SOUZA

PROF. DR. LUIS HENRIQUE DA SILVA POERSCH (Coordenador do PPGAq)

Sumário

"Quem costuma vir de onde eu sou às vezes não tem motivos pra seguir.
Mas eu sei que vai, que o sonho te traz, coisas que te faz, prosseguir.
Irmão, você não percebeu que você é o único representante do seu sonho na face da Terra?
Se isso não fizer você correr, chapa, eu não sei o que vai,
Levanta e anda, vai
Somos maior, nos basta só: Sonhar, seguir!"
Emicida e Rael da Rima

Agradecimento

Gostaria de agradecer a todos que de alguma forma contribuíram para o desenvolvimento e conclusão deste trabalho.

Ao professor César S. B. Costa pela orientação, amizade e ensinamentos nesses 4 anos de convívio.

À professora Eliana Badiale-Furlong por toda a disponibilidade, amizade, conhecimento e orientação.

À todos dos laboratórios de Biotecnologia de Halófitas (BTH), Ecologia Vegetal Costeira (Ecoveco) e Micotoxinas e Ciência de Alimentos (LAMCA). Além de todos os professores, estudantes e funcionários da PPG Aquicultura.

Á Raíza e todos os meus familiares por tudo durante esses quatro anos!!!

RESUMO GERAL

O objetivo desta tese foi avaliar os efeitos da irrigação com água salina no crescimento, produtividade e composição química da biomassa de um grupo selecionado de seis halófitas brasileiras, além de testar suas capacidades antioxidantes e potenciais nutritivos na dieta do camarão marinho *Litopenaeus vannamei*. De forma a caracterizar a composição química das halófitas selecionadas, a extração de compostos fenólicos assistida por ultrassom foi otimizada (tempo de extração e poder ultrasônico) por planejamento composto central utilizando biomassa do aspargo marinho *Salicornia neei* cultivado com efluente de carcinicultura (capítulo I). As condições ótimas (315 W por 30 minutos), também aplicadas em outras halófitas, farelo de soja e arroz, resultaram num aumento de até 30% do conteúdo de compostos fenólicos extraídos. No Capítulo II foi verificado que os compostos fenólicos das halófitas apresentaram perfil de ácidos fenólicos e do flavonoide quercetina diversificado, mas todas as espécies apresentaram alta atividade antioxidante. Entretanto, algumas características inerentes às halófitas (como elevando conteúdo de metais) podem favorecer o comportamento pró-oxidante dos extratos das plantas. No Capítulo III foi determinado que dois novos genótipos (BTH1 e BTH2) de *S. neei* apresentaram alta tolerância ao estresse salino (até 769 mM NaCl) e distintas respostas cromáticas e de produção de metabólitos bioativos contra o estresse foto-oxidativo gerado pela salinidade. O genótipo BTH2 mostrou um maior desempenho, conteúdo (e diversidade) de ácidos fenólicos e capacidade antioxidante do que BTH1 quando produzidos no campo com efluentes de carcinicultura. O Capítulo IV mostra que a inserção da biomassa do genótipo BTH2 do aspargo marinho (*S. neei*) na ração de juvenis do *L. vannamei* não afeta a sobrevivência e o desempenho zootécnico dos animais. Adicionalmente, a inserção de até 30% de S. *neei* reduziu os níveis de peroxidação lipídica e alterou a coloração (tendendo ao vermelho e ao amarelo) da carne do camarão. No geral, as halófitas brasileiras estudadas apresentam um grande potencial para serem utilizadas na alimentação e em produtos à saúde de animais e homens. Este potencial foi particularmente demonstrado pelo genótipo BTH2 de *S. neei*, que demonstrou elevada produtividade com efluente salino de aquicultura.

Palavras-chave: Efeito da salinidade na composição da biomassa; Compostos bioativos; Efluente de aquicultura; Ração para *Litopenaeus vannamei*.

GENERAL ABSTRACT

This thesis aimed to evaluate the effects of saltwater irrigation on growth, productivity and biomass chemical composition of a selected group of six Brazilian halophytes, as well as to test the antioxidant and nutritive potential of their biomass in the diet of the marine shrimp *Litopenaeus vannamei*. In order to characterize the chemical composition of the selected halophytes, the ultrasound-assisted extraction of free phenolic compounds (FPC) was optimized (extraction time and ultrasonic power) by rotational central composite design using *Salicornia neei* biomass grown with shrimp effluent (Chapter I). Optimized conditions (315 W for 30 minutes) were also applied to other halophytes, soybean meal and rice, resulting in an increase up to 30% of the FPC content. Chapter II reports that the FPC content of halophytes shows a diverse profile of phenolic acids and the flavonoid quercetin, but all species showed high antioxidant activities. However, some characteristics inherent to halophytes (such as elevated metal content) may favour the pro-oxidant behaviour of plant extracts. In Chapter III it was determined that two new genotypes (BTH1 and BTH2) of *S. neei* show high tolerance to salt stress (up to 769 mM NaCl) and distinct chromatic responses and production of bioactive metabolites against the oxidative stress generated by salt stress. Both genotypes displayed high antioxidant activity. The BTH2 genotype exhibited a higher performance, content (and diversity) of phenolic acids and antioxidant capacity than BTH1 when grown in the field with shrimp effluent. Chapter IV shows that the insertion of the biomass of the BTH2 genotype of the sea asparagus *S. neei* in the juvenile ration of the marine shrimp *L. vannamei* does not affect the survival and growth performance of the animals. Additionally, insertion of up to 30% of *S. neei* reduced lipid peroxidation levels and change colour (tending towards red and yellow) of shrimp meat. In general, the studied Brazilian halophytes have great potential to be used for feeding and insertion in care products of humans and animals. This potential was particularly shown by the BTH2 genotype of the marine asparagus *S. neei*, which showed high productivity when grown irrigated with saline effluent of the aquaculture.

Keywords: Salinity effect in biomass composition; Bioactive compounds; Aquaculture effluent; *Litopenaeus vannamei* diet.

1. Introdução Geral

Apesar da aquicultura possibilitar a produção de grandes volumes de alimento com alta qualidade nutricional, principalmente proteína animal (FAO, 2016), o rápido crescimento deste sistema de produção nas últimas décadas veio atrelado a inúmeros episódios de impacto socioambientais em diversas regiões ao redor do mundo (Ribeiro et al., 2014).

Visando tornar a atividade ambientalmente mais amigável e melhorar os rendimentos econômicos, inúmeros pacotes tecnológicos foram desenvolvidos com o intuito de diminuir o volume de água utilizado e/ou a concentração de contaminantes no efluente. O tratamento e a reciclagem da água utilizada na aquicultura diminui a taxa de renovação e podem gerar "subprodutos" a serem consumidos pela própria espécie criada (*e.g.*, tecnologia de bioflocos – BFT) (Emerenciano et al., 2013; Wasielesky et al., 2006) e/ou fontes alternativas de renda para o aquicultor, como o método multitrófico. Neste último, espécies de diferentes níveis tróficos são produzidas conjuntamente (Irisarri et al., 2015) com objetivo de melhorar o aproveitamento do alimento ofertado. Algas e plantas superiores aquáticas também podem ser associadas a sistemas multitróficos (ou a outros sistemas de cultivo) para fitorremediação de compostos dissolvidos, além da biomassa vegetal produzida ter alta qualidade nutricional (Costa, 2006; Webb et al., 2013; Pinheiro et al., 2017).

Para inserção de plantas superiores em sistemas de aquicultura com água salgada é necessária à utilização de espécies halófitas, ou seja, fanerógamas que possuem adaptações anatomorfológicas e fisiológicas capazes de tolerar elevadas salinidades. Halófitas apresentam origem polifilética, são encontradas em manguezais, marismas e desertos salgados, sendo que o nível de halofitismo (*i.e.*, o grau de tolerância a altas salinidades) varia consideravelmente entre as espécies (Rozema e Schat, 2013). Algumas plantas são consideradas "halófitas extremas" por crescerem em solos alagados com água do mar (35 g NaCl L^{-1} ≈ 52 dS m^{-1}; condutividade elétrica de decisiemens por metro) e eventualmente hipersalinos (Costa et al., 2006; Duarte et al., 2013). A utilização de espécies halófitas em tratamentos de efluentes salinos (e.g. Pinheiro et al., 2017) e como novos cultivares da agricultura com águas salinas (Glenn

et al., 2013; Rozema e Schat, 2013; Ventura et al., 2011; Costa & Herrera, 2016) já é uma realidade.

Para a maioria das plantas cultivadas ("cultivares"), a salinização da água de irrigação é considerada severa e restritiva quando a condutividade elétrica é superior a 3,0 dS m^{-1}, ou seja, cerca de 2,0 g NaCl L^{-1}, pois o estresse salino favorece inúmeros estresses secundários, como estresse osmótico, iônico e oxidante. A elevada concentração de sais no meio extracelular gera o estresse osmótico, onde as células tendem a perder água para o meio, ou "absorver" íons salinos (*e.g.* Na). A elevação de íons salinos no citosol gera o estresse iônico, onde a concentração de alguns destes íons pode se tornar tóxica a célula. Já o estresse oxidativo é gerado principalmente quando as plantas tendem a fechar os estômatos com o objetivo de diminuir a perda de água. Com isso, a entrada de moléculas de gás carbônico é prejudicada e os elétrons gerados na fotofosforilação pela ação da radiação solar não são totalmente aproveitados para formar moléculas de glicose, gerando espécies reativas de oxigênio (EROs) (Hasegawa e Bressan, 2000; Amirul-Alam et al., 2015).

Para lidar com estes problemas, as halófitas desenvolveram ao longo de milhões de anos de evolução complexos mecanismos de tolerância à salinidade, envolvendo a produção e o acúmulo nas células de uma série de compostos responsáveis por ajustes iônicos, osmótico, sinalização e defesa antioxidante (Duarte et al., 2013; Flowers e Colmer, 2008; Rozema e Schat, 2013). Quanto maior o estresse salino, maior ativação de mecanismos de tolerância e consequente maior produção de compostos anti-estresses.

Vários destes compostos produzidos pelas halófitas, como alguns ácidos graxos, compostos fenólicos e carotenoides, possuem atividades antioxidantes, anti-inflamatória e outras propriedades bioativas quando consumidos por animais e/ou pelo homem (Costa, 2006; Costa et al., 2014, 2006; D'Oca et al., 2012; Duarte et al., 2013; Barreira et al., 2017). Indícios de atividades antibacteriana, anti-helmíntica e antiviral (e.g. profilaxia da "white spot syndrome virus" – WSSV) já foram detectadas na biomassa de algumas espécies halófitas (Katiki et al., 2013; Ksouri et al., 2009; Shamsuddin et al., 2013; Tomazali Junior et al., 2017). Adicionalmente, algumas espécies halófitas podem ser empregadas para a geração de óleos e de subprodutos ricos em proteínas e

carboidratos, que podem ser utilizados na alimentação e/ou suplementação de peixes, ruminantes, galinhas, ovelhas e humana (Glenn et al., 2013; Miyamoto et al., 1996; Norman et al., 2013; Ríos-Durán et al., 2013; Swingle et al., 1996).

A inserção de componentes vegetais em rações na aquicultura vem sendo objeto de inúmeras pesquisas, principalmente a substituição da farinha e/ou óleo de peixes (Barroso et al., 2014) por soja (Yu et al., 2013), milho (Yu et al., 2015), algas (Hussein et al., 2013) e biomassa de halófitas (Belal and Al-Dosari, 1999; Ríos-Durán et al., 2013). Devido à composição singular das halófitas, influenciada pelo estresse salino, a inserção das mesmas em rações poderá melhorar a saúde e, consequentemente, os índices zootécnicos de animais aquáticos. O aporte de compostos bioativos, dentre os quais antioxidantes (e.g. compostos fenólicos), favorece o sistema de defesa-desintoxicante (Chiu et al., 2010; Wang et al., 2006), o que pode levar a diminuição do gasto energético com o sistema de defesa e aumento do crescimento do animal. Adicionalmente, a melhor sanidade animal, devido à presença de compostos bioativos em sua ração, pode melhorar a qualidade da carne ou mesmo aumentar o tempo de prateleira do produto (Ouraji et al., 2009; Badiale-Furlong, 2018).

O Brasil possui uma rica flora de halófitas costeiras com um grande potencial biotecnológico e econômico para incorporação em sistemas de aquicultura salina multitrófica. Contudo, poucos estudos da fisiologia e composição química das halófitas costeiras brasileiras são encontrados na literatura (e.g. Bertin et al., 2014; Costa et al., 2006), particularmente aqueles que permitam uma avaliação dos efeitos da concentração salina de cultivo em seu crescimento e produção de biomassa. Além disso, fatores ambientais, como condições físico-químicas da água salgada utilizada para a irrigação e o clima local (particularmente, o estresse hídrico e a incidência de radiação solar), podem influenciar a composição química (Costa et al., 2006; Duarte et al., 2013; Rozema e Schat, 2013), a taxa de crescimento (Ventura e Sagi, 2013), valor nutricional (Ventura et al., 2015; Bertin et al. 2014) e o investimento reprodutivo das halófitas (Costa, 2011). Logo, o desenvolvimento de qualquer pacote tecnológico que vise a produção e a utilização de biomassas de halófitas, deve conter a avaliação de como as condições ambientais locais e as diferenças nas características climáticas regionais influenciam na composição química das plantas.

Nesta tese foi avaliado a composição de compostos bioativos e a atividade anti-oxidante da biomassa de seis espécies de halófitas nativas, com ampla distribuição na costa brasileira. Algumas populações naturais destas espécies vêm sendo estudadas e/ou encontram-se disponíveis no germoplasma do Laboratório de Biotecnologia de Halófitas (BTH; Instituto de Oceanografia, Universidade Federal do Rio Grande-FURG, Rio Grande, RS). Foram estudadas: duas árvores, *Schinus terebinthifolius* Raddi (nome popular - Aroeira-vermelha) e *Myrsine parvifolia* A. DC (Capororoca); duas plantas anuais que crescem a partir de sementes, *Apium graveolens* L. (Aipo-do-banhado) e *Vigna luteola* (Jacq.) Benth (Feijão-da-praia); além de duas herbáceas com capacidade de multiplicação vegetativa de seus caules, *Paspalum vaginatum* Sw. (Capim-arame-da-praia) e *Salicornia neei* (Lag.) (aspargo marinho). Estas halófitas costeiras já possuem variedades comercializadas (*e.g.*, aroeira-vermelha – sementes "Brazilian pepper"; salsão ou aipo), tiveram seu potencial bioativo-nutricional preliminarmente quantificado (aroeira-vermelha, Santos et al., 2007; capim-arame-da-praia, DesRochers et al., 2009; feijão-da-praia, Bosch, 2004; aspargo-marinho, Costa et al., 2014; D'Oca et al., 2012) ou pertencem a gêneros que possuem espécies correlatadas cujo potencial econômico vem sendo avaliado (*Myrsine*, atividade antiinflamatória e antihelmintica; Ahmad et al., 2011; Githiori et al., 2002).

1.1. Estrutura da tese

De forma a caracterizar a composição química das halófitas selecionadas inicialmente, no Capítulo I, a metodologia de extração para compostos fenólicos foi otimizada, visando uma maior eficácia, rapidez e menor consumo de reagentes. Neste contexto, metodologia assistida por ultrassom foi avaliada para a extração de compostos fenólicos, uma vez que a mesma pode ser aplicada para inúmeros compostos bioativos. Foram testadas diferentes frequências do ultrassom e tempos de extração através de um planejamento experimental. Amostras de folhas e caules das cinco halófitas selecionadas foram obtidas de diferentes localidades. Um modelo quadrático foi aplicado com um ponto ótimo de extração obtido para *S. neei* e posteriormente empregado nas demais halófitas. Os resultados das extrações assistidas por ultrassom foram comparados com extrações em agitador orbital. A origem dos fenólicos

associados as matrizes halófitas foi então discutida em termos de sua quantidade total e das formas livres ou complexadas.

No Capítulo II foi realizada a quantificação dos teores foliares dos diferentes ácidos fenólicos e do flavonoide quercetina das seis halófitas selecionadas, utilizando extratos etanoicos. A metodologia de extração assistida por ultrassom otimizada foi empregada. As atividades anti-oxidante dos extratos contra radicais 2,2-Diphenyl-1-picrylhydrazyl (DPPH) e enzimas peroxidase foram também quantificadas. A eficiência do método cromatográfico de quantificação dos fenólicos é comentada. Os perfis fenólicos encontrados foram muito diversos entre as halófitas, mas todos com a predominância de compostos com conhecidas propriedades bioativas, destacando-se os ácidos clorogênico, *p*-hidroxibenzóico e cafeico, além da vanilina. Os extratos diluídos de todas as espécies apresentaram alta atividade antioxidante frente aos dois radicais livres testados (DPPH e H_2O_2), entretanto extratos de algumas espécies em maiores concentrações mostraram atividades pró-oxidantes. Estas respostas são discutidas em relação à característica da matriz vegetal halófita e a alta capacidade destas plantas sequestrarem metais em seus tecidos (Flowers & Colmer, 2015), que poderiam catalisar atividades pró-oxidantes de ácidos fenólicos e flavonoides (Eghbaliferiz & Iranshahi, 2016; Strlič et al., 2002).

No Capítulo III, em estufa foi avaliado o papel da salinidade na composição fenólica do aspargo marinho *S. neei*. Esta planta com ampla distribuição geográfica e comprovada capacidade de ser produzida com águas e efluentes em diversas salinidades, teve o efeito da adaptação ao estresse salino no seu crescimento e sua composição de metabólitos avaliados. Esta análise foi feita em dois genótipos contrastantes (BTH1 e BTH2), selecionados no Instituto de Oceanografia da FURG, no programa de melhoramento genético da espécie. O crescimento e a composição de metabólitos destes dois genótipos também foram avaliados em plantas cultivadas em um canteiro aberto irrigado com efluente da carcinicultura. Ambos os genótipos mostraram alta tolerância ao aumento de salinidade (até 769 mM NaCl) e distintas respostas cromáticas e de produção de metabólitos bioativos contra o estresse foto-oxidativo gerado pela salinidade. O genótipo BTH2 mostrou melhor desempenho no cultivo de campo e maior diversidade e quantidades individuais de ácidos fenólicos foram

produzidas pelos dois genótipos no campo. As plantas de aspargo marinho irrigadas com efluentes salinos também apresentaram uma alta capacidade antioxidante contra radicais DPPH, maior do que quando cultivadas em estufa, e de que vegetais comerciais cultivados com água doce. O potencial de produção do aspargo marinho irrigado com água salgada como alimento funcional é também discutido.

O Capítulo IV avalia da inserção da biomassa do genótipo BTH2 do aspargo marinho na ração para o crescimento de juvenis do camarão marinho *L. vannamei* em um experimento com inclusões de 0, 10 e 30% desta planta. Os resultados mais promissores mostraram uma sobrevivência de 100% dos camarões em todos os tratamentos, e que os parâmetros de desempenho zootécnico não diferiram estatisticamente entre as dietas. Adicionalmente, a inserção de até 30% de *S. neei* reduziu os níveis de peroxidação lipídica e alterou a coloração (tendendo ao vermelho e ao amarelo) da carne do camarão. Estes resultados são discutidos em relação à alta porcentagem de ácidos graxos saturados (destacadamente os ácidos palmítico e esteárico) no farelo de *S. neei* e da opção de substituir óleos de peixe ricos em ácidos graxos poli-insaturados nas dietas testadas.

1.2. Hipóteses

As seguintes hipóteses foram testadas no desenvolvimento da Tese:

H1) As seis espécies de halófitas selecionadas apresentarão quantidade total e composição de compostos bioativos variada, devido à diversidade filogenética do grupo e aos diferentes graus de adaptação ao estresse salino (Capítulos I e II);

H2) As biomassas dos genótipos BTH1 e BTH2 de *S. neei* apresentarão compostos fenólicos, pigmentos e atividade antioxidante diretamente relacionadas ao aumento da salinidade, bem como os valores destes atributos serão maiores no campo do que quando cultivados em estufa, sob condições mais amenas de disponibilidade hídrica e insolação (Capítulos III);

H3) A boa qualidade nutricional, capacidade antioxidante e presença de metabólitos bioativos permitirão a incorporação de altos teores do farelo do aspargo marinho *S. neei* na dieta do camarão marinho *L. vannamei*, com a manutenção de índices zootécnicos e melhoria no tempo de prateleira da carne do camarão (Capítulos IV).

1.3. Referências bibliográficas

Ahmad, B., Azam, S., Bashir, S., 2011. Anti-inflammatory activity and a new compound isolated from aerial parts of *Myrsine africana*. African J. Biotechnol. 10, 8465–8470. doi:10.5897/AJB11.115

Amirul Alam, M., Juraimi, a. S., Rafii, M. Y., Hamid, a. a., Aslani, F., & Alam, M. Z. (2015). Effects of salinity and salinity-induced augmented bioactive compounds in purslane (*Portulaca oleracea* L.) for possible economical use. *Food Chemistry*, *169*, 439–447. https://doi.org/10.1016/j.foodchem.2014.08.019

Badiale-Furlong, E., 2018. Transformações bioquímicas dos tecidos animais empregados na alimentação humana. Capítulo 12 In: Lajolo, F. M. & Mercadante, A. Z. (Eds.), Química e Bioquímica dos Alimentos. Coleção ciência, tecnologia, engenharia de alimentos e nutrição (CCTEAN), Volume 2. Ed. Atheneu. São Paulo.

Barreira, L., Resek, E., Rodrigues, M. J., Rocha, M. I., Pereira, H., Bandarra, N., … Custódio, L., 2017. Halophytes: Gourmet food with nutritional health benefits? *Journal of Food Composition and Analysis*, *59*, 35–42. https://doi.org/10.1016/j.jfca.2017.02.003

Barroso, F.G., de Haro, C., Sánchez-Muros, M.-J., Venegas, E., Martínez-Sánchez, A., Pérez-Bañón, C., 2014. The potential of various insect species for use as food for fish. Aquaculture 422-423, 193–201. doi:10.1016/j.aquaculture.2013.12.024

Belal, I.E.H., Al-Dosari, M., 1999. Replacement of Fish Meal with Salicornia Meal in Feeds for Nile Tilapia *Oreochromis niloticus*. J. World Aquac. Soc. 30, 285–289. doi:10.1111/j.1749-7345.1999.tb00877.x

Bertin, R. L., Gonzaga, L. V., Borges, G. D. S. C., Azevedo, M. S., Maltez, H. F., Heller, M., Micke, G. A., Tavares, L. B. B., Fett, R., 2014. Nutrient composition and, identification/quantification of major phenolic compounds in *Sarcocornia ambigua* (Amaranthaceae) using HPLC–ESI-MS/MS. Food Res. Int. 55, 404–411. doi:10.1016/j.foodres.2013.11.036

Bosch, C.H., 2004. Vigna luteola (Jacq.) Benth [WWW Document]. Grubben, G.J.H Denton, O.A. (Editors). PROTA (Plant Resour. Trop. Africa), Wageningen, Netherlands. URL http://www.prota4u.org/search.asp

Chiu, S.T., Hsieh, S.L., Yeh, S.P., Jian, S.J., Cheng, W., Liu, C.H., 2010. The increase of immunity and disease resistance of the giant freshwater prawn, *Macrobrachium rosenbergii* by feeding with selenium enriched-diet. Fish Shellfish Immunol. 29, 623–629. doi:10.1016/j.fsi.2010.06.012

Costa, C.S.B., 2006. A Salicórnia - Uma planta que pode ser utilizado em cultivo integrado com o camarão. Panoroma da Aquicultura 16, 28–33.

Costa, C.S.B., 2011. Restoration of coastal salt marshes in Brazil using native salt marsh plants, in: Greipsson, S. (Ed.), Restoration Ecology. Jones & Barlett Learning, Sudbury, MA, United State of America, p. 408.

Costa, C.S.B., Armstrong, R., Detrés, Y., Koch, E.W., Bertiller, M., Beeskow, a, Neves, L.S., Tourn, G.M., Bianciotto, O. A, Pinedo, L.B., Blessio, a Y., San Roman, N., 2006. Effect of ultraviolet-B radiation on salt marsh vegetation: Trends of the genus *Salicornia* along the Americas. Photochem. Photobiol. 82, 878–86. doi:10.1562/2005-10-30-RA-729

Costa, C.S.B., Vicenti, J.R.M., Morón-Villareyes, J.A., Caldas, S., Cardoso, L. V, Freitas, R.F., D'Oca, M.G.M., 2014. Extraction and characterization of lipids from *Sarcocornia ambigua* meal: a halophyte biomass produced with shrimp farm effluent irrigation. An. Acad. Bras. Cienc. 86, 935–943. doi:10.1590/0001-3765201420130022

Costa, C. S. B., & Herrera, O. B., 2016. Halophytic Life in Brazilian Salt Flats: Biodiversity, Uses and Threats. In M. A. Khan, B. Boër, M. Ozturk, M. Clüsener-Godt, B. Gul, & S.-W. Breckle (Eds.), *Sabkha Ecosystem V : The Americas* (1ª, pp. 11–27). Berlin: Springer International Publishing. https://doi.org/10.1007/978-3-319-27093-7_2

D'Oca, M.G.M., Morón-Villarreyes, J.A., Lemões, J.S., Costa, C.S.B., 2012. Fatty acids composition in seeds of the South American glasswort *Sarcocornia ambigua*. An. Acad. Bras. Cienc. 84, 865–870. doi:10.1590/S0001-37652012005000044

DesRochers, D.W., McWilliams, S.R., Silbernagle, M.D., Reed, J.M., 2009. Macronutrient profiles of wetland plants consumed by the Hawaiian Moorhen (*Gallinula chloropus sandvicensis*). Wetlands 29, 845–853. doi:10.1672/08-189.1

Duarte, B., Santos, D., Marques, J.C., Caçador, I., 2013. Ecophysiological adaptations of two halophytes to salt stress: photosynthesis, PS II photochemistry and anti-oxidant feedback--implications for resilience in climate change. Plant Physiol. Biochem. 67, 178–88. doi:10.1016/j.plaphy.2013.03.004

Eghbaliferiz, S., & Iranshahi, M., 2016. Prooxidant activity of polyphenols, flavonoids, anthocyanins and carotenoids: Updated review of mechanisms and catalyzing metals. *Phytotherapy Research*, (March), 1379–1391. https://doi.org/10.1002/ptr.5643

Emerenciano, M., Gaxiola, G., Cuzon, G., 2013. Biofloc technology (BFT): A review for aquaculture application and animal food industry, in: Matovic, M.D. (Ed.), Biomass Now-Cultivation and Utilization. InTech, p. 460. doi:10.5772/3437

FAO, 2016 The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200 pp.

Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. New Phytol. 179, 945–63. doi:10.1111/j.1469-8137.2008.02531.x

Githiori, J.B., Höglund, J., Waller, P.J., Baker, R.L., 2002. Anthelmintic activity of preparations derived from *Myrsine africana* and *Rapanea melanophloeos* against the nematode parasite, *Haemonchus contortus*, of sheep. J. Ethnopharmacol. 80, 187–191. doi:10.1016/S0378-8741(02)00030-2

Glenn, E.P., Anday, T., Chaturvedi, R., Martinez-Garcia, R., Pearlstein, S., Soliz, D., Nelson, S.G., Felger, R.S., 2013. Three halophytes for saline-water agriculture: An oilseed, a forage and a grain crop. Environ. Exp. Bot. 92, 110–121. doi:10.1016/j.envexpbot.2012.05.002

Hasegawa, P., Bressan, R., 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51, 463–499. doi:10.1146/annurev.arplant.51.1.463

Hussein, E.E.-S., Dabrowski, K., El-Saidy, D.M.S.D., Lee, B.-J., 2013. Enhancing the growth of Nile tilapia larvae/juveniles by replacing plant (gluten) protein with algae protein. Aquac. Res. 44, 937–949. doi:10.1111/j.1365-2109.2012.03100.x

Irisarri, J., Fernández-Reiriz, M.J., Labarta, U., Cranford, P.J., Robinson, S.M.C., 2015. Availability and utilization of waste fish feed by mussels *Mytilus edulis*

in a commercial integrated multi-trophic aquaculture (IMTA) system: A multi-indicator assessment approach. Ecol. Indic. 48, 673–686. doi:10.1016/j.ecolind.2014.09.030

Katiki, L.M., Ferreira, J.F.S., Gonzalez, J.M., Zajac, A.M., Lindsay, D.S., Chagas, A.C.S., Amarante, A.F.T., 2013. Anthelmintic effect of plant extracts containing condensed and hydrolyzable tannins on *Caenorhabditis elegans*, and their antioxidant capacity. Vet. Parasitol. 192, 218–227. doi:10.1016/j.vetpar.2012.09.030

Ksouri, R., Falleh, H., Megdiche, W., Trabelsi, N., Mhamdi, B., Chaieb, K., Bakrouf, A., Magné, C., Abdelly, C., 2009. Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents. Food Chem. Toxicol. 47, 2083–2091. doi:10.1016/j.fct.2009.05.040

Miyamoto, S., Glenn, E.P., Olsen, M.W., 1996. Growth, water use and salt uptake of four halophytes irrigated with highly saline water. J. Arid Environ. 32, 141–159. doi:10.1006/jare.1996.0013

Norman, H.C., Masters, D.G., Barrett-Lennard, E.G., 2013. Halophytes as forages in saline landscapes: Interactions between plant genotype and environment change their feeding value to ruminants. Environ. Exp. Bot. 92, 96–109. doi:10.1016/j.envexpbot.2012.07.003

Ouraji, H., Shabanpour, B., Kenari, A.A., Shabani, A., Nezami, S., Sudagar, M., Faghani, S., 2009. Total lipid, fatty acid composition and lipid oxidation of Indian white shrimp (*Fenneropenaeus indicus*) fed diets containing different lipid sources. J. Sci. Food Agric. 89, 993–997. doi:10.1002/jsfa.3545

Pinheiro, I., Arantes, R., Espírito Santo,, C. M., Vieira, F. N., Lapa, K. R., Gonzaga, L. V., Fett, R., Barcelos-Oliveira, J. L. Seiffert, W. Q., 2017. Production of the halophyte *Sarcocornia ambigua* and Pacific white shrimp in an aquaponic system with biofloc technology. Ecol. Eng. 100,261-267. 10.1016/j.ecoleng.2016.12.024

Ribeiro, L.F., Souza, M.M. de, Barros, F., Hatje, V., 2014. Desafios da carcinicultura: aspectos legais, impactos ambientais e alternativas mitigadoras. Rev. Gestão Costeira Integr. 14, 365–383. doi:10.5894/rgci453

Ríos-Durán, M.G., Valencia, I.R., Ross, L.G., Martínez-Palacios, C. A., 2013. Nutritional evaluation of autoclaved *Salicornia bigelovii* Torr. seed meal supplemented with varying levels of cholesterol on growth, nutrient utilization and survival of the Nile

tilapia (Oreochromis niloticus). Aquac. Int. 21, 1355–1371. doi:10.1007/s10499-013-9638-5

Rozema, J., Schat, H., 2013. Salt tolerance of halophytes, research questions reviewed in the perspective of saline agriculture. Environ. Exp. Bot. 92, 83–95. doi:10.1016/j.envexpbot.2012.08.004

Santos, M.R.A., Lima, R.A., Silva, A.G., Fernandes, C. de F., Lima, D.K.S., Sallet, L.A.P., Teixeira, C.A.D., Facundo, V.A., 2007. Atividade inseticida do óleo essencial de *Schinus terebinthifolius* Raddi sobre *Acanthoscelides obtectus* Say e *Zabrotes subfasciatus* Boheman, Boletim de Pesquisa e Desenvolvimento (INFOTECA-E). Porto Velho, Rondônia.

Shamsuddin, A.A., Najiah, M., Suvik, A., Azariyah, M.N., Kamaruzzaman, B.Y., Effendy, A.W., Akbar John, B., 2013. Antibacterial properties of selected mangrove plants against *Vibrio* species and its cytotoxicity against *Artemia salina*. World Appl. Sci. J. 25, 333–340. doi:10.5829/idosi.wasj.2013.25.02.688

Strlič, M., Radovič, T., Kolar, J., & Pihlar, B., 2002. Anti- and prooxidative properties of gallic acid in fenton-type systems. *Journal of Agricultural and Food Chemistry*, *50*(22), 6313–6317. https://doi.org/10.1021/jf025636j

Swingle, R.S., Glenn, E.P., Squires, V., 1996. Growth performance of lambs fed mixed diets containing halophyte ingredients. Anim. Feed Sci. Technol. 63, 137–148. doi:10.1016/S0377-8401(96)01018-8

Tomazelli Junior, O., Kuhn, F., Mendonça Padilha, P.J., Mota Vicente, L.R., Winckler da Costa, S., Corrêa da Silva, B., Dias Schleder, D., Boligon, A.A., Scapinello, J., Nunes Nesi, C., Dal Magro, J., De Lamo Castellví, S., 2017. Effect of *Cynodon dactylon* extract on white spot virus-infected *Litopenaeus vannamei*. Aquac. Int. 25, 1107–1122. doi:10.1007/s10499-016-0101-2

Ventura, Y., Sagi, M., 2013. Halophyte crop cultivation: The case for *Salicornia* and *Sarcocornia*. Environ. Exp. Bot. 92, 144–153. doi:10.1016/j.envexpbot.2012.07.010

Ventura, Y., Wuddineh, W.A., Myrzabayeva, M., Alikulov, Z., Khozin-Goldberg, I., Shpigel, M., Samocha, T.M., Sagi, M., 2011. Effect of seawater concentration on the productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia*

halophytes as leafy vegetable crops. Sci. Hortic. (Amsterdam). 128, 189–196. doi:10.1016/j.scienta.2011.02.001

Ventura, Y., Eshel, A., Pasternak, D., Sagi, M., 2015. The development of halophyte-based agriculture: past and present. Annals of Botany 115, 529-540. 10.1093/aob/mcu173

Wang, W.N., Wang, Y., Wang, A.L., 2006. Effect of supplemental l-ascorbyl-2-polyphosphate (APP) in enriched live food on the immune response of *Penaeus vannamei* exposed to ammonia-N. Aquaculture 256, 552–557. doi:10.1016/j.aquaculture.2006.02.017

Wasielesky, W., Atwood, H., Stokes, A., Browdy, C. L., 2006. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. Aquaculture 258, 396-403. 10.1016/j.aquaculture.2006.04.030

Webb, J.M., Quintã, R., Papadimitriou, S., Norman, L., Rigby, M., Thomas, D.N., Le Vay, L., 2013. The effect of halophyte planting density on the efficiency of constructed wetlands for the treatment of wastewater from marine aquaculture. Ecol. Eng. 61, 145–153. doi:10.1016/j.ecoleng.2013.09.058

Yu, D.-H., Gong, S.-Y., Yuan, Y.-C., Luo, Z., Lin, Y.-C., Li, Q., 2013. Effect of partial replacement of fish meal with soybean meal and feeding frequency on growth, feed utilization and body composition of juvenile Chinese sucker, Myxocyprinus asiaticus (Bleeker). Aquac. Res. 44, 388–394. doi:10.1111/j.1365-2109.2011.03043.x

Yu, H.-B., Gao, Q.-F., Dong, S.-L., Wen, B., Hou, Y.-R., Ning, L.-G., 2015. Utilization of corn meal and extruded soybean meal by sea cucumber *Apostichopus japonicus* (Selenka): Insights from carbon stable isotope analysis. Aquaculture 435, 106–110. doi:10.1016/j.aquaculture.2014.09.036

2. Objetivos

2.1. Objetivo geral

Avaliar os efeitos da irrigação com água salina no crescimento, na produtividade e na composição química da biomassa de um grupo selecionado de seis halófitas brasileiras, além de testar suas capacidades antioxidantes e potenciais nutritivos na dieta do camarão marinho *L. vannamei*.

2.2. Objetivos específicos

i) Avaliar a variação na composição bioquímica e de atividades antioxidantes de seis espécies selecionadas de halófitas brasileiras coletadas em seus ambientes de ocorrência e frente a diferentes condições de estresse salino (H1 e H2);

ii) Avaliar a capacidade nutricional, através de índices zootécnicos (*e.g.* taxa de conversão alimentar e taxa de crescimento específico) e vida útil da carne após despesca (*e.g.* análises de cor, textura, pH, peroxidação lipídica e quantificação de bases voláteis totais) do camarão marinho (*L. vannamei*) submetido a diferentes porcentuais de inserção da biomassa do aspargo marinho *S. neei* na sua dieta (H3).

Capítulo 1

Artigo aceito para publicação nos Anais da Academia Brasileira de Ciências.

Free phenolic compounds extraction from Brazilian halophytes, soybean and rice bran by ultrasound-assisted and orbital shaker methods

Manuel M de Souza[1*], Bibiana da Silva[2], César S B Costa[1], Eliana Badiale-Furlong[2]

[1]Laboratório de Biotecnologia de Halófitas (BTH), Instituto de Oceanografia (IO), Universidade Federal do Rio Grande – FURG, Av. Itália km 8, 96203-900 Rio Grande, RS, Brazil;

[2]Laboratório de Micotoxinas e Ciência de Alimentos, Programa de Pós-Graduação em Engenharia e Ciência de Alimentos, Escola de Química e Alimentos (EQA), FURG, Av. Itália km 8, 96203-900 Rio Grande, RS, Brazil.

Keywords: Sea asparagus; Celery; Brazilian pepper; Sea water crops.

Running title: Extraction of phenolic compounds in *S. ambigua*

Academy Section: Agrarian Sciences

Email address: bibianaengenheira@hotmail.com (B da Silva); costacsb@hotmail.com (CSB Costa); dqmebf@furg.br (E Badiale-Furlong); mcsouza@furg.br (*corresponding author MM de Souza).

RESUMO:

Compostos fenólicos têm diversas funções fisiológicas em plantas, como sinalização e defesa contra patógenos e espécies reativas de oxigênio. No homem, os compostos fenólicos produzidos pelas plantas trazem inúmeros benefícios à saúde, incluindo o controle a diferentes tipos de câncer. Uma fonte de compostos fenólicos vegetais que vem ganhando destaques são as plantas halófitas. Halófitas são plantas que possuem diversas adaptações anatomorfológicas e fisiológicas para tolerar condições ambientais adversas (água e/ou solos salinos), dentre as quais a produção de compostos bioativos para osmorregulação e combate aos estresses oxidativo, como os compostos fenólicos. A produção desses compostos conferem as halófitas inúmeras propriedades bioativas, como capacidade antioxidante, antibactericida e antiviral. Cultivos comerciais de plantas halófitas com água salina ou efluente salino de aquicultura já são uma realidade em diversos países para produção de óleo das sementes e alimentação humana e animal (e.g. aspargos marinhos, halófitas dos gêneros *Salicornia* e *Sarcocornia*). No Brasil, a *Sarcocornia ambigua* já vem sendo produzida a nível experimental com águas de poços salinos e efluentes de aquicultura, com elevada concentração de compostos fenólicos e boa aceitabilidade nas dietas humana e animal. Porém, apesar dos seus benefícios à saúde humana, a extração de compostos fenólicos de matrizes vegetais ainda utiliza-se de métodos morosos que necessitam de elevado volume de solventes tóxicos (e.g. metanol). Além disso, a elevada concentração de material lignocelulósico pode reduzir a eficiência de extração, sendo necessário a utilização de métodos físicos mais abruptos, como métodos de extração assistido por ultrassom (UAE, do inglês ultrasound assisted extraction). Desta forma, buscar métodos mais rápidos e eficientes para extração de compostos fenólicos de halófitas e outras matrizes vegetais torna-se uma estratégia interessante para diversos setores da sociedade (química, farmacêutica e saúde). Sendo assim, este trabalho teve como objetivo otimizar a extração assistida por ultrassom (UAE) de compostos fenólicos livres (FPC) do aspargo marinho *S. ambigua* por meio de um planejamento composto central rotacional, variando os parâmetros: tempo de extração (1, 9, 30, 51, e 60 min) e poder das ondas ultrassônicas (100, 150, 275, 400, e 450 W). As amostras de *S. ambigua* utilizadas nesta otimização foram obtidas de cultivos realizados com efluente da carcinicultura na cidade de Aracati, Ceará. As condições ótimas de extração (315 W durante 30 min, que resultou em uma intensidade

acústica de 5, 89 W m^{-2}) melhoram a recuperação de compostos fenólicos em *S. ambigua* em 30%, quando comparado ao método em agitador orbital. As condições otimizadas da UAE também foram aplicadas em outras halófitas brasileiras (*Apium graveolens*, *Myrsine parvifolia*, *Paspalum vaginatum* e *Schinus terebinthifolius*), farelo de arroz e soja. Com exceção de *P. vaginatum*, soja e farelo de arroz, os resultados da extração UAE melhoraram a extração de FPC entre 18 e 29%. Além de melhor desempenho analítico, o método UAE otimizado é mais rápido que o agitador orbital, proporcionando menor exposição do analista ao solvente extrator e aplicável em matrizes com diferentes composições. Também foi demonstrado que as espécies halófitas mostraram-se boas fontes naturais de FPC e este foi o primeiro trabalho a reportar o conteúdo de FPC em *M. parvifolia* and *P. vaginatum*.

Palavras-chave: Aspargo marinho; Aipo; Pimenta brasileira; Cultivo com água do mar.

ABSTRACT

In several countries halophytes are commercially cultivated in low saline or even with irrigated with seawater, as well as with saline aquaculture effluent, like a sea asparagus *Sarcocornia ambigua*, that show a biotechnological potential for bioactive compounds production. However their recovery from matrix is sometimes inefficient because the lignocellulosic materials difficult the solvent action when drastic conditions are not applied. The ultrasound-assisted extraction (UAE) was optimized by a central composite rotational design for recovery free phenolic compounds (FPC) from the sea asparagus *S. ambigua*. Optimum conditions were validate and compared with orbital shaker extraction for *S. ambigua,* other Brazilian halophytes (*Apium graveolens*, *Myrsine parvifolia*, *Paspalum vaginatum*, and *Schinus terebinthifolius*), soybean and rice bran. Except for *P. vaginatum*, soybean and rice bran, UAE yielded 18-29% higher FPC than that of the orbital shaker. Besides this analytical performance UAE method optimized is faster than the orbital shaker, providing shorter exposure of the analyst to the extractor solvent and applicable in matrices with different compositions. It was also demonstrated that halophytes species showed to be good natural sources of FPC in a better way as soybean and rice bran. This work was the first to report FPC in *M. parvifolia* and *P. vaginatum*.

Keywords: Sea asparagus; Celery; Brazilian pepper; Seawater crops.

1. Introduction

Phenolic compounds are synthesized by several plants and they play a structural role, working on signalling and defense, and against oxidative damage (Garcia-Salas et al. 2010). For humans, the control of free radicals by phenolic compounds from vegetable sources can have positive effects on health, including control of different types of cancer (Stanković et al. 2015).

High contents of phenolic compounds are found in coastal halophytes (salt tolerant plants), which produce these compounds in order to survive under stressful conditions (high salt concentration, periodical submersion, frequently high levels of soil contaminants; Costa et al. 2006; Rozema and Schat 2013; Flowers and Colmer 2015; Mishra et al. 2015). Their phenolic contents are directly proportional to the intensity of environmental stress during growth (Costa et al. 2006; Ventura et al. 2011; Bertin et al. 2014) and several of these compounds produced by halophytes have bioactive properties, detected *in vitro* and *in vivo* (e.g. antibacterial, and antiviral) (Stanković et al. 2015; Wang et al. 2016).

In several countries some halophytes are commercially cultivated in low saline or even irrigated with seawater, as well as with saline aquaculture effluent (e.g. Amaranthaceae as *Salicornia* and *Sarcocornia* species), for seed oil production, animal food and human diet (Glenn et al. 2013; Rozema and Schat 2013; Costa et al. 2014). There are few studies on the amount of phenolic compounds, as well as on the composition and/or content of individual phenolic acids and flavonoids in commercial halophytes (Bertin et al. 2014; Wang et al. 2016). Due to the rapid increase on the demand for natural bioactive substances, there is a need for further phytochemical and pharmacological characterization of halophytes by an efficient procedure with a good recovery and few damages to chemical compounds structure (Stanković et al. 2015).

There are several methods for phenolic compounds extraction. Among the most frequently reported method is the extraction by orbital shaker (Scaglioni et al. 2014; Schmidt et al. 2014). However, the high amount of sample mass, type (e.g. methanol) and the large solvent volume required make this extraction method slow, dangerous and expensive. Ultrasound-assisted extractions (UAE) are mainly based on micro-streaming and sonochemical effects. In general, are employed baths and probe-type systems UAE.

Probe can deliver a higher ultrasonic power than ultrasonication bath (up to 100 times more), and is considered a more effective technique (Santos et al. 2009). Typically, ultrasonication bath present power and wave frequency fixed (Wang et al. 2016), which could limit the technique performance. Thus UAE with probe system can help to fill the gap of knowledge on halophytes' phenolic compounds constitution, intended to identify new sources of functional vegetables to animal and human diet.

Native halophytes in Brazil show good biomass production in their habitats and represent a biotechnological potential for bioactive compounds production, such as phenolic compounds, which has not yet been adequately explored. The perennial sea asparagus *Sarcocornia ambigua* (Michx.) Alonso & Crespo has been introduced as a new halophytic crop under irrigation with saline water and shrimp farm effluents in different climatic regions in Brazil (Costa et al. 2014; Bertin et al. 2016; Costa and Herrera 2016). Fresh and dry *S. ambigua* shoots have high nutritional quality for animal (Costa et al. 2014) and human diets (Bertin et al. 2014; Timm et al. 2015), being well accepted by Brazilians as pickle in vinegar (Timm et al. 2015). Bertin et al. (2014) have found a high phenolic acid and flavonoids content in *S. ambigua* shoots, using an orbital shaker extraction and determination by HPLC-ESI-MS/MS. Some of the compounds found in alcoholic extracts of *S. ambigua* (chlorogenic acids and quercetin, for example) seem to be responsible for high antioxidant and free radical scavenger activity.

In this work, UAE was optimized by a central composite rotational design for recovery free phenolic compounds (FPC) from sea asparagus *Sarcocornia ambigua*. Additionally, optimum conditions were validate and compared with orbital shaker extraction for *S. ambigua,* other Brazilian halophytes (*Apium graveolens*, *Myrsine parvifolia*, *Paspalum vaginatum*, and *Schinus terebinthifolius*), soybean and rice bran.

2. Materials and methods

2.1 Samples

All experiments for the UAE optimization were accomplished with cylindrical leafless shoots of *S. ambigua*. The 100 days old *S. ambigua* were harvested at ground level from a 90 m² cultivated plot in the city of Aracati (northeast Brazilian state of Ceará; 04º33'

S). Plants were spaced 25 cm apart and watered by filling up drainage ditches once a day, with 1350 L of saline effluent (40 g NaCl L^{-1}) from a *Litopenaeus vannamei* shrimp tank. Only apical branches with fertile segments (with seeds) were utilized. After harvesting, the samples were freeze dried (- 50 ºC; 72 h).

The optimized UAE procedure was applied in others halophytes with detected bioactivity. Celery *Apium graveolens* L. (antioxidant), seashore paspalum *Paspalum vaginatum* Sw. (rich in free radical scavengers), and the deciduous marsh trees *Myrsine parvifolia* A. DC. (antibacterial) and *Schinus terenbithifolius* Raddi (antioxidant and antimicrobial) (Suffredini et al. 2006; Yao et al. 2010; Uddin et al. 2012; Uliana et al. 2016), as well as soybeans and rice bran.

Seeds of *A. graveolens* were collected in the Pólvora Island salt marsh located in Rio Grande (RS, Brazil; 32°01′ S, 52°06′ W). Vegetative propagules of *P. vaginatum* were from active germplasm bank of the Laboratório de Biotecnologia de Halófitas (BTH; Instituto de Oceanografia, FURG, Rio Grande, RS, Brazil); accession 2006/010-Caravelas. Plants of these two halophytes were cultivated in commercial organic compost irrigated with freshwater for two months in the greenhouse of BTH before harvest. Fully-expanded green leaves of *M. parvifolia* and *S. terebinthifolius* were collected in the Carreiros Campus of FURG, in Rio Grande (32º04’ S, 52º09’ W). Soybeans and rice bran matrices were provided by the Brazilian Agricultural Research Corporation (EMBRAPA, Londrina, PR, Brazil) and purchased at a local shop (Rio Grande, RS, Brazil), respectively. All above cited samples were dried in oven (60 ºC; 48 h), grinded in a knives mill, sieved through 0.50 mm, and maintained in freezer at -20 ºC, until determinations were obtained.

2.2 Reagent and analytical solutions

All solutions were prepared from analytical reagent grade chemicals (> 95 per cent of purity). Ethanol, zinc sulfate, barium hydroxide, sodium carbonate and copper sulphate were provided by Synth (Brazil). Sodium potassium tartrate was provided by Vetec (Sigma-Aldrich, Brazil) and Folin-Ciocalteu phenol reagent (2N) from Dynamics (Brazil).

2.3 Experimental design for UAE optimization

The central composite rotational design (CCRD) was applied to optimize UAE of FPC from *S. ambigua*. CCRD 2^2 was used, with 11 trials (Table 1), including three replicates at the center point. The effect of extraction time (1, 9, 30, 51, and 60 min) and power of ultrasonic waves (100, 150, 275, 400, and 450 W) variables were investigated. Samples (250 mg) with addition of 15 mL of ethanol 80 per cent were submitted to extraction in focused ultrasound (Ultrasonic disruptor Ecosonics, Ultronique, QR500, 60 Hz, 20 kHz, 500 W, Brazil). Then, each extract was clarified with 2.50 mL of barium hydroxide 0.1 M and 2.5 mL of zinc sulfate 5 per cent, centrifuged (Centrifuge Eppendorf 5804 R, Germany) at 2990 x g and filtrated for analysis. The final volume was 25 mL. Ethanol is a widely used solvent for extraction of polar FPC (Krishnaswamy et al. 2013; Wang et al. 2016) and the levels of variables studied in this experimental design were chosen based on preliminary tests (data not shown).

Acoustic intensity was determined for the optimal conditions using following formula (González-Centeno et al., 2015):

$$I = P / \pi r^2 \quad (1)$$

$$P = mC_p (\Delta T / t) \quad (2)$$

where "I" is the acoustic intensity (W/cm²), "P" is the ultrasonic power applied (W), "r" is the radius of the probe (0.2 cm), "m" is the mass of solvent (12.5 10^{-3} kg), "C_p" is the specific heat capacity of the solvent (4180 J kg^{-1} K^{-1}), "T" is the temperature (K), and "t" is the sonication time (s).

2.3.1 Validation of the proposed optimized UAE method

The parameters assessed for validation were limit of detection (LD) and limit quantification (LQ) repeatability and accuracy. LD and LQ were estimated in 0.14 and 0.45 µg mL^{-1}, respectively. Limit of detection was estimated by three times standard deviation of

blanks divided by the angular coefficient, and limit of quantification by five times standard deviation divided by the angular coefficient (AOAC 2002).

Accuracy was obtained in triplicate and measured by the percentage of recovery of fortified *S. ambigua* shoot samples, with three levels of a standard of gallic acid (intermediate values of the standard curve of gallic acid), in amounts of 416, 520 and 728 μg g^{-1} of the sample (dry weight), by equation 1. For complete evaporation of the solvent, solutions of the three amounts of gallic acid diluted in methanol were prepared and placed on the samples to be analyzed 24 hours before analysis. Samples with methanol and without gallic acid were prepared simultaneously as control. The repeatability was tested by the precision on the assays performed to recovery, on the same day, by assessing the relative standard deviation (%RSD) of the results obtained.

$$\text{Extraction recovery (\%)} = \frac{\text{calculated concentration of gallic acid}}{\text{experimental concentration of gallic acid}} \times 100 \qquad (3)$$

2.4 Extraction by orbital shaker

Samples (250 mg) with addition of 10 mL of ethanol 80 per cent were submitted to extraction in an orbital shaker (Tecnal TE-420, Brazil) at 180 rpm, for 60 min, at 25.0 °C. Then, agitation was stopped for 15 min, 5 mL of ethanol 80 per cent was added and agitation started again for 90 min. Extracts were clarified with 2.5 mL of barium hydroxide 0.1 M and 2.5 mL of zinc sulfate 5 per cent, centrifuged at 2990 x *g* and filtrated for posterior analysis. The final volume was 25 mL.

2.5 Quantification of FPC

Free phenolic compounds were measured by the Folin-Ciocalteu method, using a spectrophotometer (Biospectro, SP-22, Brazil) (Souza et al. 2009). Extracts (0.5 mL), distilled water (0.5 mL) and alkaline solution (4.5 mL) of sodium carbonate, copper sulphate and sodium potassium tartrate (100:1:1) were placed into a bath at 40° C for 15

min. The Folin-Ciocalteu (1:2) was added (0.5 mL) and after 10 min the absorbance was measured at 750 nm. The concentration of FPC was estimated from a standard curve of gallic acid (concentration ranging from 1.7 to 8.6 µg mL^{-1}) and expressed as milligram of gallic acid equivalents (GAE) per gram of dry weight sample (mg GAE g^{-1} dw). Measurements were performed in triplicate.

2.6 Statistical analysis

All determinations were carried out in triplicate and the results obtained were expressed as means and standard deviation. The optimal extraction conditions of FPC from *S. ambigua* were set through the construction of a quadratic polynomial model. The multiple regression model was built on the actual data of FPC response to extraction time, power ultrasound and their interaction, considering both linear and quadratic fitting. The regression model fitting was assessed by Analysis of Variance (ANOVA) and Fisher F-test at 95 per cent confidence interval. Student's "t" tests were applied to investigate the statistical significance of regression coefficients. Surface plots were employed to visualize to relationship between FPC response and experimental factors.

Student's t-test was applied to compare difference in FPC recover between UAE and shaker extractions methods for each vegetable matrix ($p < 0.05$).

3. Results and Discussion

3.1 UAE optimization for phenolic extraction from S. ambigua shoots

The Folin-Ciocalteu method is one the most utilized for quantification of free phenolic compounds (e.g. Arruda et al. 2017). Only between the years 2016 and 2017 were published more than nine thousand papers using this method for FPC determination. Although the Folin-Ciocalteu reagent is non-specific for phenolic compounds, this reaction is favored by medium alkaline (Singleton and Rossi 1965).

Concentrations of FPC in *S. ambigua* shoots ranged between 16.3 and 20.0 mg GAE g^{-1} dw in CCRD 2^2 (Table 1). The multiple regression showed that power ultrasound (linear

and quadratic term) and extraction time (quadratic term) significantly affected FPC extraction by UAE ($p < 0.05$) (see Table 2a). The power ultrasound was the factor with the highest influence (small p value of the quadratic term) in FPC concentration, with higher concentrations observed at intermediate potency levels (275 W) (Table 1). Extraction time at 30 min produced high FPC values from *S. ambigua* shoots and longer extractions led to slightly lower contents, and the interaction between tested factors had no significant effect (Table 2a). Wang et al. (2016) also found lower extractions of gallic acid under ultrasonic exposure for more than 50 min and suggested that longer ultrasonic exposure could lead to degradation of extracts (by rise in temperature) and/or lower recoveries associated with slow diffusion (increased of the solvent viscosity by evaporation).

Table 1. Central composite rotational design and results for the extraction of free phenolic compounds (FPC) from *Sarcocornia ambigua* shoot biomass.

Trials	Extraction time (min)	Power (W)	FPC (mg GAE g^{-1} dw)
1	9 (-1)	150 (-1)	17.0
2	51 (+1)	150 (-1)	17.7
3	9 (-1)	400 (+1)	19.5
4	51 (+1)	400 (+1)	19.3
5	1 (-1,41)	275 (0)	18.7
6	60 (+1,41)	275 (0)	18.6
7	30 (0)	100 (-1,41)	16.3
8	30 (0)	450 (+1,41)	17.9
9	30 (0)	275 (0)	20.0
10	30 (0)	275 (0)	19.9
11	30 (0)	275 (0)	19.8

dw – dry weight; GAE – Galic acid equivalent.

The optimized mathematical model relating extraction time (T) and ultrasound power (P) with the content of FPC in terms of significant independent factors was (equation 2):

$$Y_{FPC} = 19.9 - 0.49T^2 + 0.81P - 1.29P^2 \quad (4)$$

The relation between observed and predicted values pointed out a strong positive correlation ($R^2 = 0.93$; see Table 2b) and the regression results showed that the model is significant (F-value = 29.89, $p < 0.001$; Table 2b) and can be used to predict FPC content from *S. ambigua* shoots obtained by UAE.

The response surface (Figure 1) as well as raw data (Table 1) show higher FPC content in central point's tested. Through the first partial assessment, the Y_{FPC} equation detected the optimum values of 30 min and 315 W, for extraction time and ultrasound power, respectively (integer values were utilized because of the machine settings). The optimal conditions found in the intermediated parameters values generate a maximum temperature during the 30 min sonication time of 55.0 ºC (328.15 ºK) and a consequent acoustic intensity (I) of 5.89 W cm^{-2}. Acoustic/ultrasound intensity is proportional to the amplitude of ultrasound (and ultrasonic power applied is proportional the amplitude). In low acoustic intensity, are not observed cavitation. Cavitation bubbles are created by ultrasound cross of the medium. When collapsing bubbles are generating zones of high pressure and temperature, occur extraction process. However, elevated acoustic intensity (or power) can lead to rapid deterioration of the compounds (Santos et al. 2009). Under these optimal conditions of extraction, the experimental values detected FPC concentration in *S. ambigua* shoots were 24.4 ± 4.21 mg GAE g^{-1} dw. Considering the value predicted by equation 2; this FPC concentration detected presents a coefficient of variation of 17.7 per cent.

Table 2. Multiple regression coefficients and analysis of variance (ANOVA) of the effects of extraction time (T; min) and ultrasound power (P; W) on the extraction of free phenolic compounds (FPC; mg GAE g^{-1} dry-weight) from *S. ambigua* shoot biomass.

(a) Coefficients

Variable	Coefficient	SE	*t-value*	*p-value*
Constant	19.90	0.25	78.90	< 0.0001
T (L)	0.05	0.31	0.29	0.7815
T (Q)	-0.49	0.37	-2.67	0.0445
P (L)	0.81	0.31	5.52	0.0034
P (Q)	-1.29	0.37	-6.99	0.0009
T (L) x P (L)	-0.21	0.44	-0.94	0.3881

(b) ANOVA

Source	SS	*df*	MS	*F-value*	*p value*
Model	14.6	3	4.88	29.9	0.0002
Error	1.14	7	0.16		
R^2	0.93				

L – Linear; Q – Quadratic; SE – Standard error of coefficient; SS – Sum of Square; *df* – degree of freedon; MS – Mean Square.

3.1.1 *Validation of the proposed method*

The accuracy was evaluated by the mean of recovery percentages of gallic acid (equation 1) from the fortified *S. ambigua* samples by the optimized UAE method. According to AOAC (2002), the recovery limits acceptable are a function of the concentration and the purpose of the analysis. For the three levels studied (416, 520 and 728 µg g^{-1}dw), the recovery percentages were 101, 97.4 and 100 per cent respectively,

which means that the method presents the expected accuracy, approximately 99.5 per cent. Precision was calculated by the coefficient of variation (CV) of the three replicates of each level tested. The CVs of 416, 520 and 728 µg gallic acid g^{-1} dw were 2.8, 10, and 8.0 per cent, respectively, showing that the precision of this method is acceptable (<15 per cent) (AOAC 2002).

3.2 *Extraction methods and phenolic compounds recovery*

The UAE with optimized parameters to *S. ambigua* shoots was applied in other halophyte matrices, soybeans and rice bran to obtain FPC contents, and these values were compared with the orbital shaker extraction (Figure 2). Except for *P. vaginatum* shoots, on average, the UAE method yielded significantly higher amounts of FPC, 18 - 29 per cent, than the shaker method for all halophytes studied. Halophytes have, to a larger or lesser degree, convergent physiological and anatomic-morphological adaptations to salt stress, such as high lignification of their tissues and ions, and organic osmolites accumulation in their cells (Jbir et al. 2001; Flowers and Colmer 2015; Lutts and Lefèvre 2015; Slama et al. 2015). These characteristics are partially responsible for the form in which the phenols associate with halophyte matrices (i.e. free and bound phenols). Our results suggest that halophytes possess interfering compounds and/or are rich in more bound phenols, which passive processes of FPC extraction (dependent on the solvent capacity of penetration into the cell), such as orbital shaker, have difficulty to access and extract from the plant sample. The active process of UAE is more efficient for matrices with more bound phenols concentrations, since the cavitation produced in the solvent by the focused ultrasonic waves (and consequently high temperature and pressure) allowed extraction of greater contents of phenolic compounds (Garcia-Salas et al. 2010).

Higher UAE efficiency in halophyte matrices may also be associated with the reduced analytical time exposure to the solvent effect, and consequent reduced time of more labile phenolic compounds exposure to oxidative degradation. Higher recovery of ultrasound-assisted extractions of numerous bioactive compounds, such as phenols, than traditional methods (e.g. orbital shaker) have been previously reported (Abid et al.

2014; Wang et al. 2016). Our results show that the utilization of UAE is an important asset to a proper evaluation of the halophytes potential as a source of FPC and probably of other bioactive compounds. Further studies are necessary in order to clarify why UAE improves phenols extraction from halophytes.

Among the matrices analyzed, halophyte plants presented the higher contents of phenolic compounds. The highest FPC yield for *S. ambigua* shoots (25.0 ± 0.17 mg GAE g^{-1} dw) was obtained with UAE method, whereas shaker extraction yielded 18.8 ± 0.81 mg GAE g^{-1}dw. Moreover, FPC concentrations found were greater than blackberry, red raspberry, strawberry, blueberry, cherry (De Souza et al. 2014), grape seeds (Krishnaswamy et al. 2013), and even others halophytes (*Crithmum maritimum* L. and *Inula crithmoïdes* L.; Jallali et al. 2014). Many previous works have shown the phenolic extraction potential and the main phenolic compounds of *Sarcocornia* species (Ventura et al. 2011; Bertin et al. 2014; Stanković et al. 2015).

In this work it was detected an average FPC concentration of 16.5 ± 0.09 mg GAE g^{-1} dw for *Apium graveolens* shoots by UAE (Shaker = 13.8 ± 0.07 mg GAE g^{-1} dw), a halophyte widely used in human food and a member of the *Apiaceae* family, like coriander, parsley and anise. This content of a marsh originated accession was 10-folds higher than values found by Yao et al. (2010) in commercial celery cultivars (averages ranging from 1.12-1.74 mg GAE g^{-1} for *A. graveolens*).

Both the Brazilian pepper *S. terebinthifolius* (Wheeler et al. 2001) and *M. parvifolia* (Ribeiro and Costa 2015) are widely spread trees in Brazil, occurring in the inland border of coastal wetlands and also in drier habitats of coastal plains. Brazilian pepper seeds are traditionally used as condiment and its leaves are utilized in folk medicine for numerous health treatments (e.g. respiratory problems and rheumatism) (Uliana et al. 2016). Though our study disclosed a high FPC average content in leaves of *S. terebinthifolius* (UAE = 14.8 ± 0.02 mg GAE g^{-1} dw; Shaker = 12.5 ± 0.10 mg GAE g^{-1} dw), Uliana et al. (2016) founded a much higher value in leaves (221.63 mg GAE g^{-1} dw). This difference may be associated to the extraction method employed, since Uliana et al. (2016) utilized high leaf mass (60 g) and volume of solvent (400 mL) and longer extraction time (seven days). In our study *M. parvifolia* presented 16.1 ± 0.04 mg GAE

g-1 dw by UAE (Shaker = 12.6 ± 0.06 mg GAE g-1 dw). To the best of our knowledge there are no reports about concentration of phenolic compounds in *M. parvifolia*.

Distinctively, UAE and orbital shaker methods yield average values of FPC very similar for shoots of grass *P. vaginatum* (UAE = 12.0 ± 0.34 mg GAE g^{-1} dw; Shaker = 12.2 ± 0.22 mg GAE g^{-1} dw), soybean (UAE = 2.29 ± 0.18 mg GAE g^{-1} dw; Shaker = 2.38 ± 0.21 mg GAE g^{-1} dw) and rice bran (UAE = 7.10 ± 0.03 mg GAE g^{-1} dw; Shaker = 7.02 ± 0.33 mg GAE g^{-1} dw) ($p > 0.05$; see Figure 2). We believe that these three matrices were particularly rich in free phenols, reachable by both extraction methods. In the review of *P. vaginatum*, Lonard et al. (2015) pointed out that this grass is a true halophyte with enhanced growth at low salinity, and osmotic adjustments enhanced by the accumulation of potassium ions and the amino acid proline in its tissue. According to these authors there is no report available about bioactive compounds or medical uses of *P. vaginatum*, although many ecotypes of this species are resistant to insects that normally cause severe damage to lawns and other types of recreational sites in the coastal zone. Thus, this species is probably rich in chemical defense compounds, such as phenols. To our knowledge this work was the first to report FPC in *P. vaginatum*.

Although soybean and rice bran have high bound phenols concentrations, the industrial processing and storing of grains may release bound phenols (Scaglioni et al. 2014; Xiao et al. 2015). The significant phenolic content in soybean and rice bran (Scaglioni et al. 2014; Ali et al. 2015) is often associated to defense mechanisms against disease and pests (Nicholson and Hammerschmidt 1992). In soybeans, the content of phenolic compounds is responsible for the *flavor* (Alu'datt et al. 2013) and genetic modification may change the composition of the seed by increased defense mechanism where the phenolic compounds are included (Ladics et al. 2014). The soy cultivar used in our study was not genetically modified and showed intermediate FPC contents among values found by Alu'datt et al (2013) (1.87 mg g^{-1}). In comparison with others published FPC contents in rice bran by different extraction methods (Scaglioni et al. 2014), our UAE and orbital shaker results ranked in the mid-upper range of obtained values.

4. Conclusions

In this work, ultrasound-assisted extraction (UAE) was optimized for *S. ambigua* shoots, although halophytes may present high concentration of interfering factors (e.g. Na^+, Cl^-, K^+, metals, lignification, others) in the extraction and detection of FPC. The experimental design tested was efficient to find the optimal conditions of the extraction. The proposed method proved to be accurate and precise, with recuperation percentages around 99.5 per cent and precision lower than 20 per cent of variation.

This work was the first to report FPC in *M. parvifolia* and *P. vaginatum*. All halophytes species showed to be good natural sources of FPC. The optimized UAE method proved to be a better extraction procedure than the orbital shaker method for *S. ambigua* and most of others halophyte matrices tested (*A. graveolens*, *M. parvifolia* and *S. terenbithifolius*). Furthermore, the optimized UAE method is faster than the orbital shaker method, providing shorter exposure of the analyst to the extractor solvent.

5. References

Abid M, Jabbar S, Wu T, Hashim MM, Hu B, Lei S and Zeng X. 2014. Sonication enhances polyphenolic compounds, sugars, carotenoids and mineral elements of apple juice. Ultrason Sonochem 21:93–97. doi: 10.1016/j.ultsonch.2013.06.002

Ali NM, Yeap S-K, Yusof HM, Beh B-K, Ho W-Y, Koh S-P, Abdullah MP, Alitheen NB and Long K. 2015. Comparison of free amino acids, antioxidants, soluble phenolic acids, cytotoxicity and immunomodulation of fermented mung bean and soybean. J. Sci. Food Agric. doi:10.1002/jsfa.7267

Alu'datt MH, Rababah T, Ereifej K and Alli I. 2013. Distribution, antioxidant and characterisation of phenolic compounds in soybeans, flaxseed and olives. Food Chem. 139, 93–99. doi:10.1016/j.foodchem.2012.12.061

AOAC. 2002. Validation of chemical methods for dietary. Supplements and botanicals, Guidelines for single laboratory.

Arruda HS, Pereira GA and Pastore GM. 2017. Optimization of extraction parameters of total phenolics from *Annona crassiflora* Mart. (Araticum) fruits using

response surface methodology. Food Anal Methods 10:100–110. doi: 10.1007/s12161-016-0554-y

Bertin RL, Gonzaga LV, Borges GDSC, Azevedo MS, Maltez HF, Heller M, Micke GA, Tavares LBB and Fett R. 2014. Nutrient composition and, identification/quantification of major phenolic compounds in *Sarcocornia ambigua* (Amaranthaceae) using HPLC–ESI-MS/MS. Food Res. Int. 55, 404–411. doi:10.1016/j.foodres.2013.11.036

Bertin RL, Maltez HF, Gois JS, Borges DLG, Borges GSC, Gonzaga LV and Fett R. 2016. Mineral composition and bioaccessibility in *Sarcocornia ambigua* using ICP-MS. J. Food Compos. Anal. 47, 45–51. doi:10.1016/j.jfca.2015.12.009

Costa CSB, Armstrong R, Detrés Y, Koch EW, Bertiller M, Beeskow A, Neves LS, Tourn GM, Bianciotto O, Pinedo LB, et al. 2006. Effect of ultraviolet-B radiation on salt marsh vegetation: Trends of the genus *Salicornia* along the Americas. Photochem. Photobiol. 82, 878–86. doi:10.1562/2005-10-30-RA-729

Costa CSB and Herrera OB. 2016. Halophytic life in Brazilian salt flats: Biodiversity, uses and threats., in: Khan MA, Boër B, Ozturk M, Clüsener-Godt M, Gul B and Breckle SW. (Eds.), Sabkha Ecosystem V : The Americas. Springer International Publishing, Berlin, pp. 11–27. doi:10.1007/978-3-319-27093-7_2

Costa CSB, Vicenti JRM, Morón-Villareyes JA, Caldas S, Cardoso LV, Freitas RF and D'Oca MGM. 2014. Extraction and characterization of lipids from *Sarcocornia ambigua* meal: a halophyte biomass produced with shrimp farm effluent irrigation. An. Acad. Bras. Cienc. 86, 935–943. doi:10.1590/0001-3765201420130022

De Souza VR, Pereira PAP, da Silva TLT, de Oliveira Lima LC, Pio R and Queiroz F. 2014. Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. Food Chem. 156, 362–368. doi:10.1016/j.foodchem.2014.01.125

Flowers TJ and Colmer TD. 2015. Plant salt tolerance: adaptations in halophytes. Ann Bot 115:327–331. doi: 10.1093/aob/mcu267

Garcia-Salas P, Morales-Soto A, Segura-Carretero A and Fernández-Gutiérrez A. 2010. Phenolic-compound-extraction systems for fruit and vegetable samples. Molecules 15, 8813–8826. doi:10.3390/molecules15128813

Glenn EP, Anday T, Chaturvedi R, Martinez-Garcia R, Pearlstein S, Soliz D, Nelson SG and Felger RS. 2013. Three halophytes for saline-water agriculture: An oilseed, a forage and a grain crop. Environ. Exp. Bot. 92, 110–121. doi:10.1016/j.envexpbot.2012.05.002

González-Centeno MR, Comas-Serra F, Femenia A, Rosselló C and Simal S. Effect of power ultrasound application on aqueous extraction of phenolic compounds and antioxidant capacity from grape pomace (*Vitis vinifera* L.): Experimental Kinetics and modeling. Ultrason. Sonochem. 26, 506-514. http://dx.doi.org/10.1016/j.ultsonch.2014.05.027

Jallali I, Zaouali Y, Missaoui I, Smeoui A, Abdelly C and Ksouri R. 2014. Variability of antioxidant and antibacterial effects of essential oils and acetonic extracts of two edible halophytes: *Crithmum maritimum* L. and *Inula crithmoïdes* L. Food Chem. 145, 1031–1038. doi:10.1016/j.foodchem.2013.09.034

Jbir N, Chaïbi W, Ammar S, Jemmali A and Ayadi A. 2001. Root growth and lignification of two wheat species differing in their sensitivity to NaCl, in response to salt stress. Comptes Rendus l'Académie des Sci. - Ser. III - Sci. la Vie 324, 863–868. doi:10.1016/S0764-4469(01)01355-5

Krishnaswamy K, Orsat V, Gariépy Y and Thangavel K. 2013. Optimization of Microwave-Assisted Extraction of Phenolic Antioxidants from Grape Seeds (*Vitis vinifera*). Food Bioprocess Technol 6:441–455. doi: 10.1007/s11947-012-0800-2

Ladics GS, Budziszewski GJ, Herman RA, Herouet-Guicheney C, Joshi S, Lipscomb EA, McClain S and Ward JM. 2014. Measurement of endogenous allergens in genetically modified soybeans - Short communication. Regul Toxicol Pharmacol 70:75–79. doi: 10.1016/j.yrtph.2014.06.006

Lonard RI, Judd FW and Stalter R. 2015. Biological flora of coastal dunes and wetlands: *Paspalum vaginatum* Sw. J Coast Res 31:213–223. doi: 10.2112/JCOASTRES-D-14-00022.1

Lutts S and Lefèvre I. 2015. How can we take advantage of halophyte properties to cope with heavy metal toxicity in salt-affected areas? Ann Bot 115:509–28. doi: 10.1093/aob/mcu264

Mishra A, Patel MK and Jha B. 2015. Non-targeted metabolomics and scavenging activity of reactive oxygen species reveal the potential of *Salicornia brachiata* as a functional food. J Funct Foods 13:21–31. doi: 10.1016/j.jff.2014.12.027

Nicholson RL and Hammerschmidt R. 1992. Phenolic compounds and their role in disease resistance. Annu Rev Phytopathol 30:369–389. doi: 10.1146/annurev.py.30.090192.002101

Ribeiro JNS and Costa CSB. 2015. The effect of temperature regulation on seed germination of the tropical tree *Myrsine parvifolia* A. DC near its southern limit. South African J Bot 98:128–133. doi: 10.1016/j.sajb.2015.02.012

Rozema J and Schat H. 2013. Salt tolerance of halophytes, research questions reviewed in the perspective of saline agriculture. Environ Exp Bot 92:83–95. doi: 10.1016/j.envexpbot.2012.08.004

Santos HM, Lodeiro C and Capelo-Martínez J-L. 2009. The Power of Ultrasound. In: Ultrasound in Chemistry. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp 1–16

Scaglioni PT, de Souza TD, Schmidt CG and Badiale-Furlong E. 2014. Availability of free and bound phenolic compounds in rice after hydrothermal treatment. J Cereal Sci 60:526–532. doi: 10.1016/j.jcs.2014.08.005

Schmidt CG, Gonçalves LM, Prietto L, Hackbart HS and Furlong EB. 2014. Antioxidant activity and enzyme inhibition of phenolic acids from fermented rice bran with fungus *Rizhopus oryzae*. Food Chem 146:371–377. doi: 10.1016/j.foodchem.2013.09.101

Singleton VL and Rossi JAJ. 1965. Colorimetry of total phenolics with phosphomolybdic-phossphotungstic acid reagents. Am J Enol Vitic 16:144–158.

Slama I, Abdelly C, Bouchereau A, Flowers T and Savoure A. 2015. Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. Ann Bot 115:433–447. doi: 10.1093/aob/mcu239

Souza MM de, Recart VM, Rocha M da, Cipolatti EP and Badiale-Furlong E. 2009. Study on the extracting conditions of phenolic compounds from onion (*Allium cepa* L.). Rev Inst Adolfo Lutz 68:192–200.

Stanković MS, Petrović M, Godjevac D and Stevanović ZD. 2015. Screening inland halophytes from the central balkan for their antioxidant activity in relation to total phenolic compounds and flavonoids: Are there any prospective medicinal plants? J Arid Environ 120:26–32. doi: 10.1016/j.jaridenv.2015.04.008

Suffredini IB, Paciencia ML, Varella AD and Younes RN. 2006. Antibacterial activity of Brazilian Amazon plant extracts. Brazilian J Infect Dis 10:400–402. doi: http://dx.doi.org/10.1590/S1413-86702006000600008

Timm TG, Júnior AAS, Bertin RL and Tavares LBB. 2015. Processamento de conservas de *Sarcocornia perennis*. Agropecuária Catarinense 28:97–102.

Uddin K, Juraimi AS, Ismail MR, Hossain A, Othman R and Abdul Rahim A. 2012. Physiological and growth responses of six turfgrass species relative to salinity tolerance. Sci World J 2012:Article ID 905468, 10 pages. doi: 10.1100/2012/905468

Uliana MP, Fronza M, da Silva AG, Vargas TS, de Andrade TU and Scherer R. 2016. Composition and biological activity of Brazilian rose pepper (*Schinus terebinthifolius* Raddi) leaves. Ind Crops Prod 83:235–240. doi: 10.1016/j.indcrop.2015.11.077

Ventura Y, Wuddineh WA, Myrzabayeva M, Alikulov Z, Khozin-Goldberg I, Shpigel M, Samocha TM and Sagi M. 2011. Effect of seawater concentration on the productivity and nutritional value of annual Salicornia and perennial Sarcocornia halophytes as leafy vegetable crops. Sci Hortic (Amsterdam) 128:189–196. doi: 10.1016/j.scienta.2011.02.001

Wang X, Cai C and Li X-M. 2016. Optimal Extraction of Gallic Acid from *Suaeda glauca* Bge. Leaves and Enhanced Efficiency by Ionic Liquids. Int J Chem Eng 2016:1–9. doi: 10.1155/2016/5217802

Wheeler GS, Massey LM and Endries M. 2001. The Brazilian peppertree drupe feeder *Megastigmus transvaalensis* (Hymenoptera: Torymidae): Florida distribution and impact. Biol Control 22:139–148. doi: 10.1006/bcon.2001.0968

Xiao Y, Wang L, Rui X, Li W, Chen X, Jiang M and Dong M. 2015. Enhancement of the antioxidant capacity of soy whey by fermentation with *Lactobacillus plantarum* B1–6. J Funct Foods 12:33–44. doi: 10.1016/j.jff.2014.10.033

Yao Y, Sang W, Zhou M and Ren G. 2010. Phenolic composition and antioxidant activities of 11 celery cultivars. J Food Sci 75:C9–C13. doi: 10.1111/j.1750-3841.2009.01392.x

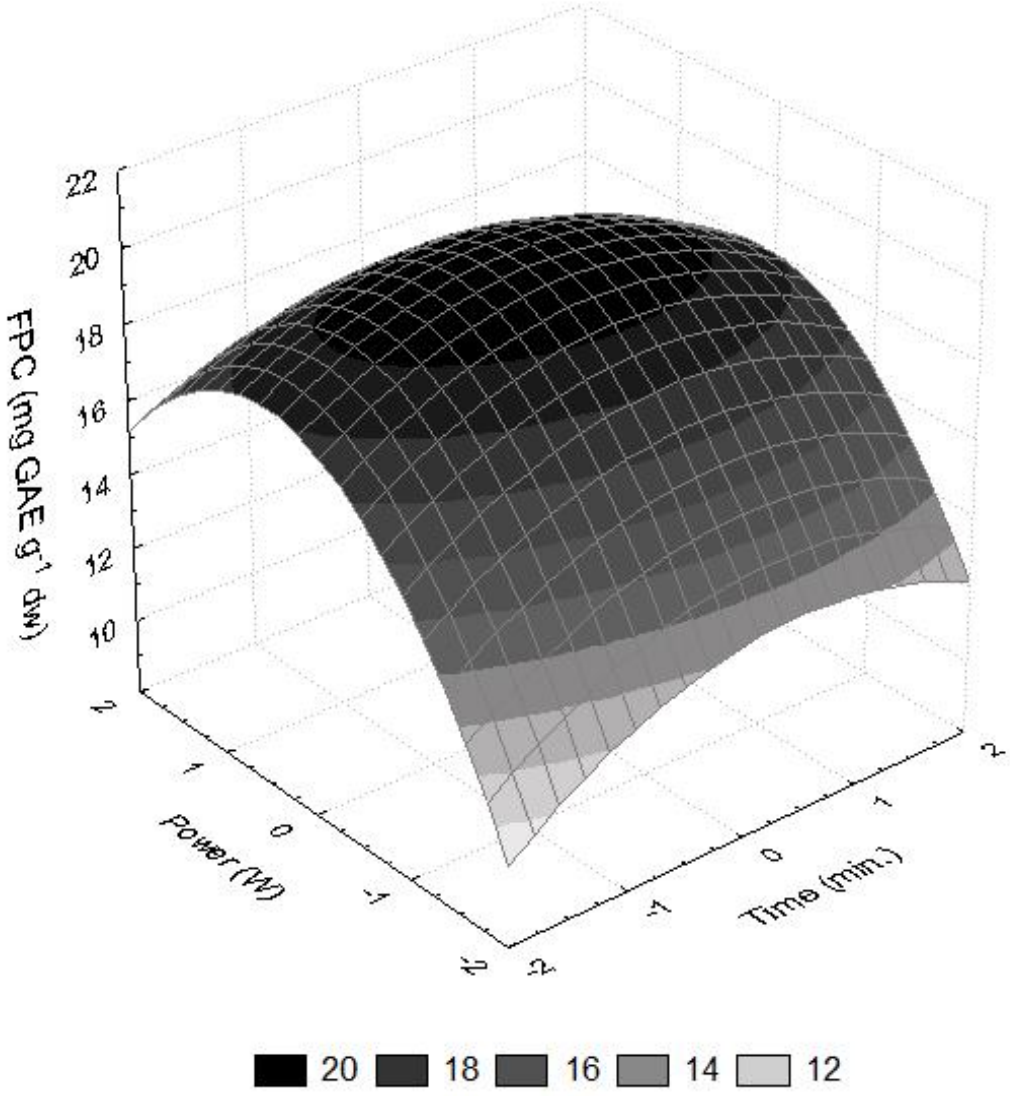


Figure 1. Surface response plot (3D) presenting the effects of extraction time (min) and ultrasound power (W) on the extraction of free phenolic compounds (FPC; mg GAE g^{-1} dry-weight) from *Sarcocornia ambigua* shoot biomass.

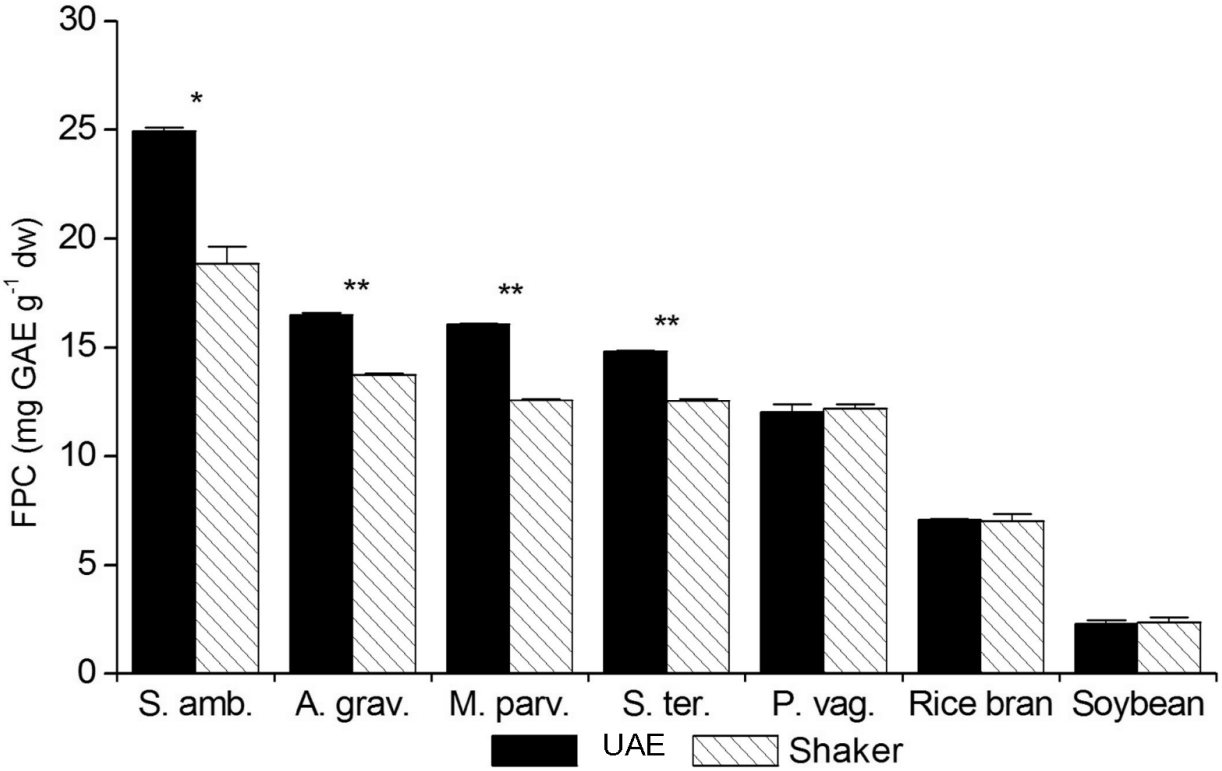


Figure 2. Average values (± standard-deviation) of free phenolic compounds (FPC) concentration (mg GAE g^{-1} dry-weight) from different matrices obtained by two extraction methods; UAE - Ultrasound assisted extraction; Shaker - orbital shaker. *$p < 0.01$; **$p < 0.001$. S. amb – *Sarcocornia ambigua*; A. gra. – *Apium graveolens*; M. par. – *Myrsine parvifolia*; S. ter. – *Schinus terebinthifolius*; P. vag. – *Paspalum vaginatum*.

Capítulo 2

Phenolic acid profile, quercetin content and antioxidant activity of six Brazilian halophytes

Manuel M de Souza[1*], Bibiana da Silva[2], Eliana Badiale-Furlong[2], César S B Costa[1]

[1]Laboratório de Biotecnologia de Halófitas (BTH), Instituto de Oceanografia (IO), Universidade Federal do Rio Grande – FURG, Av. Itália km 8, 96203-900 Rio Grande, RS, Brazil;

[2]Laboratório de Micotoxinas e Ciência de Alimentos, Programa de Pós-Graduação em Engenharia e Ciência de Alimentos, Escola de Química e Alimentos (EQA), FURG, Av. Itália km 8, 96203-900 Rio Grande, RS, Brazil.

RESUMO

Plantas halófitas são plantas tolerantes ao sal, ricas em moléculas bioativas e consumidas mundialmente por populações tradicionais e usadas para tratar uma variedade de doenças. O Brasil possui uma diversa flora halofítica, com altos teores de compostos fenólicos livres em seus tecidos e propriedades bioativas (por exemplo, atividade antioxidante e antimicrobiana). Este trabalho tem como objetivo quantificar diferentes ácidos fenólicos e quercetina e atividade antioxidante da biomassa de *Apium graveolens*, *Paspalum vaginatum*, *Myrsine parvifolia*, *Salicornia neei*, *Schinus terenbithifolius* e *Vigna luteola*. As halófitas brasileiras apresentaram elevado, e diversificado, conteúdo de ácidos fenólicos e quercetina, sendo que a soma dos teores de ácidos fenólicos apresentou teores superiores à média encontrada em frutas tropicais e vegetais tradicionais. No entanto, os ácidos fenólicos e o conteúdo de quercetina não foram correlacionados com as atividades antioxidantes. Neste trabalho, verificou-se uma relação não linear entre a concentração do extrato de halófita e atividade antioxidante radical do DPPH, o que pode estar relacionado a um efeito da matriz vegetal, possivelmente pela presença de metais e metalóides. Além disso, a atividade pró-oxidante foi detectada nos extratos de *P. vaginatum*, *S neei* e *V. luteola*. Em geral, todas as espécies apresentaram alta capacidade antioxidante, mas particularmente *S. terebinthifolius* e *M. parvifolia*, que apresentaram maior e mais rápida resposta antioxidante em ambos os métodos analisados.

Palavras-chave: Aspargo marinho; Aroeira, pró-oxidante; Efeito de matriz

ABSTRACT

Halophytes are salt tolerant plants rich in bioactive molecules and consume worldwide by traditional populations as vegetables and used to treat a variety of illnesses. Brazil has a diversify halophytic flora with high contents of free phenolic compounds in their tissues and bioactive properties (*e.g.*, antioxidant and antimicrobial activity). This work aims to quantify different phenolic acids and quercetin, and antioxidant activity of shoot/leaf biomass of *Apium graveolens*, *Paspalum vaginatum*, *Myrsine parvifolia*, *Salicornia neei*, *Schinus terenbithifolius* and *Vigna luteola*. The Brazilian halophytes showed high and diversified composition of phenolic acids and quercetin, with the sum of phenolic acids' contents showed higher than mean concentrations found in tropical fruits and traditional vegetable. But the phenolic acids and quercetin content were not correlated with antioxidant activities. In this work was verified the quadratic relationship with DPPH radical antioxidant activity, can be explained by an effect of the plant matrix, possibly because of the presence of metals and metalloids. Furthermore, prooxidant activity was detected in *P. vaginatum*, *S neei* and *V. luteola* extracts. In general, all species present high antioxidant capacity, but particularly *S. terebinthifolius* and *M. parvifolia*, which had higher and faster antioxidant answer against both free radical tested.

Keywords: Sea asparagus; Brazilian pepper leaf; Prooxidant; Matrix effects

1. Introduction

Halophytes are salt tolerant plants able to thrive irrigated with brackish water or even seawater and their shoots and roots, rich in bioactive molecules, are consume worldwide by traditional populations as vegetables and used to treat a variety of illnesses. The bioactive properties of the halophyte species and their ability for survive in the saline environment are associated to several physiological adaptations, essentials to withstand ionic, osmotic and oxidative stresses (Jithesh, Prashanth, Sivaprakash, & Parida, 2006). Although such adjustments vary among species, in general, halophytes present ions bombs (for ionic stress), salt compartmentalization in vacuole and organic osmolite production in the cytoplasm (for osmotic stress), and production of enzymatic and non-enzymatic antioxidant molecules (for oxidative stress) (Amirul Alam et al., 2015; Flowers & Colmer, 2015; Jithesh et al., 2006). As for other plants, under exposition to high salt concentrations that induce plant stomatal closure and consequent CO_2 starvation of the Calvin cycle, halophytes show increasing concentrations of reactive oxygen species (ROS) due to reduction of the electron transport chain (Jithesh et al., 2006; Miller, Suzuki, Ciftci-Yilmaz, & Mittler, 2010). ROS can be free radicals (*e.g.*, superoxide and hydroxyl radical) or non-radical forms (*e.g.*, hydrogen peroxide and oxygen singlet) (Gill & Tuteja, 2010; Jithesh et al., 2006).These molecules are naturally found in cells (signalling function, mainly) but when in high concentrations they cause damage to pigments, proteins, lipids or even DNA (Amirul Alam et al., 2015; Miller et al., 2010). The ROS defence of halophytes, and for plants in general, is based on enzymatic (e.g. superoxide dismutase, ascorbate peroxidase and glutathione reductase) and non-enzymatic components, such as ascorbate, glutathione and markedly the phenolic compounds (Amirul Alam et al., 2015; Salin, 1988; Sreenivasulu, Grimm, Wobus, & Weschke, 2000).

Phenolic compounds (*e.g.*, phenolic acids, flavonoids, and tocopherol) have diverse functional roles in plants, including plant homoeostasis and response to biotic and abiotic stresses, being the most frequently studied the resistance to pathogens and herbivores, and the protection against solar radiation (Goleniowski, Bonfill, Cusido, & Palazón, 2013; Quideau, Deffieux, Douat-Casassus, & Pouységu, 2011). Besides phenolic compounds are frequently linked with scavenger actions against ROS, they may act as prooxidant (i.e., donate electrons), having in that sense important roles in the

mechanisms of reproduction, growth and nutrition (Lattanzio et al., 2009; Quideau et al., 2011). The prooxidant role of phenolic compounds depends on many factors, such as pH, dissociation energy and ionization potential of the phenol, bioavailability, concentration and presence of metals (Eghbaliferiz & Iranshahi, 2016; Quideau et al., 2011).

In humans, phenolics have numerous biological and pharmaceutical properties, such as an anti-inflammatory, anti-mutagenic and antioxidant activities (Crozier, Jaganath, & Clifford, 2009; Goleniowski et al., 2013). The ingestion of food items rich in phenolic compounds (*e.g.*, fish, olive oil, and wine) is associated with the reduction of coronary diseases, cancer, and Alzheimer's disease (Joven et al., 2014). However, a diet with olive oil and wine cannot be available for poor families in several countries, where cash crop halophytes can offer a natural food with low cost and high quality. Additionally, phenolic compounds present in halophytes can also be an alternative for phthalates (synthetic compounds used in insecticides and cosmetics products), whose active ingredients (or their degradation products) can have long half-lives and be toxic, accumulating in the environment and generate deleterious effects on animals and humans (Boxall et al., 2012; Cotrim, Fahning, Rocha, & Hatje, 2016). For example, phthalates are responsible for respiratory and dermatological problems and suspected to cause premature puberty in humans (Cotrim et al., 2016).Phenolic acids represent thirty-three percent of the phenolic compounds consumed by humans and they divided into benzoic (*e.g.* gallic, *p*-hydroxybenzoic, protocatechuic, vanillin and syringic) and cinnamic acids (*e.g.* caffeic, chlorogenic, *p*-coumaric and ferulic acids) (Giada, 2013; Goleniowski et al., 2013; Heleno, Martins, Queiroz, & Ferreira, 2015). Flavonoids are other important phenolic compounds in the human diet and certain plant tissues stand out by their quercetin content, which shows anticancer and anti-inflammatory properties, and it is also the flavonoid with greatest capacity to eliminate ROS (Ghasemzadeh & Ghasemzadeh, 2011; Giada, 2013).

Few species of halophytes are commercially cultivated (e.g. Amaranthaceae such as the quinoa *Chenopodium quinoa*, and several species of *Salicornia* and *Sarcocornia*), but, in the coming decades, the use of halophytes may be much widely and a viable commercial alternative by increase globally the production of phenolic rich food

farming salt-affected soils, semiarid and deserts areas, which can be irrigated with saline waters or effluents of the marine aquaculture (Costa et al., 2014; Panta et al., 2014; Rozema & Schat, 2013). Brazil has a diverse halophytic flora that occupies coastal salt marshes, mangroves and inland salt flats (Costa & Herrera, 2016), but the phytochemical and pharmacological properties of native halophytes remain poorly studied. For example, biochemical studies of Brazilian halophytes *Apium graveolens* L., *Myrsine parvifolia* A. DC., *Paspalum vaginatum* Sw., *Salicornia neei* Lag. and *Schinus terenbithifolius* Raddi reported high contents of free phenolic compounds in their tissues (Bertin et al., 2014; Souza, da Silva, Costa, & Badiale-Furlong, *in press*), as well as high antioxidant activity (for *A. graveolens*, *P. vaginatum*, *S. neei* and *S. terenbithifolius*; Bertin et al., 2014; Pinheiro et al., 2017; Uddin et al., 2012; Uliana et al., 2016; Yao, Sang, Zhou, & Ren, 2010) and the presence of antibacterial substances (for *M. parvifolia*, *S. terenbithifolius* and *Vigna luteola* (Jacq.) Benth.; Shrivastava, Patra, & Tikariha, 2016; Suffredini, Paciencia, Varella, & Younes, 2006).

This work aims to determine the profiles of phenolic acids and to quantify the amount of quercetin and antioxidant activities in shoot/leaf biomass of six selected Brazilian halophytes (*A. graveolens*, *P. vaginatum*, *M. parvifolia*, *S. neei, S. terenbithifolius* and *V. luteola*). Since antioxidant-prooxidant role of phenolic compounds may depend on their interaction with the plant matrix (Eghbaliferiz & Iranshahi, 2016; Quideau et al., 2011), the antioxidant activity of halophytes was evaluated in ethanolic extracts at different concentrations through various *in vitro* antioxidant assays using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical method and the inhibition of peroxidase enzyme (PO) activity.

2. Material and methods

2.1. Samples

Six species of halophytes widely distributed along the Brazilian coast were analyzed. Plants of *A. graveolens, P. vaginatum* and *V. luteola* obtained from the germplasm of Laboratório de Biotecnologia de Halófitas (Institute of Oceanography, FURG, Rio Grande, RS, Brazil). *Apium graveolens* and *V. luteola* seeds were originally obtained in Pólvora Island salt marsh located at Patos Lagoon estuary (RS, Brazil;

32°01′ S, 52°06′ W). *Paspalum vaginatum* vegetative propagules were collected in a hipersaline salt marsh at the tropical location of Caravelas (BA, Brazil; 17º43’ S, 39º15’ W). These three plants were cultivated with a mixture (1:1) of organic compound (Humosolo Vida®) and fine beach sand, irrigated with freshwater for a total of 10 weeks in an unheated greenhouse before being harvested. *M. parvifolia* and *S. terebinthifolius* were sampled from natural populations in the city of Rio Grande, RS (32º04’ S, 52º09’ W). *Salicornia neei* samples were obtained from plants cultivated in the Brazilian state of Ceará (Aracati, CE; 4º33’ S, 37º46’ W), located at the tropical semiarid region of the country. Plants of *S. neei* were grown for 15 weeks watered once a day with saline shrimp farm effluent (mean salinity = 40.7 ± 0.5 g NaCl L^{-1}) before being harvested.

Shoots (*A. graveolens* and *P. vaginatum*) and leaves (*M. parvifolia*, *S. terebinthifolius* and *V. luteola*) of samples were dried in an oven (60 ºC; 48 h). Apical branches with fertile segments of *S. neei* were freeze dried (-50 ºC; 48h).

2.2. Chemicals

Acetone, barium hydroxide, copper sulphate, ethanol, guaiacol solution, methanol, hydrogen peroxide, sodium carbonate, sodium chloride, sodium phosphate monobasic, sodium phosphate dibasic and zinc sulphate were provided from Synth (Brazil). Sodium potassium tartrate provided by Vetec (Sigma-Aldrich, Brazil) and Folin-Ciocalteu phenol reagent (2N) provided by Dynamics (Brazil). Methanol HPLC grade and glacial acetic acid were acquired from J. T. Baker (USA) and Merck (Germany), respectively. Standard of phenolic acids (caffeic, chlorogenic, *p*-coumaric, ferulic, gallic, *p*-hydroxybenzoic, protocatechuic, syringic and vanillin), quercetin and bovine serum albumim, and DPPH (2,2-Diphenyl-1-picrylhydrazyl) was obtained from Sigma-Aldrich (Brazil)

2.3. Extraction

All extractions were carried out in triplicate using ultrasound assisted extraction (UAE) (Souza et al., *in press*). Samples (250 mg) with addition of 15 mL of ethanol 80

per cent were submitted to extraction in focused ultrasound (acoustic intensity of 5.82 W cm^{-2}; Ultrasonic disruptor Ecosonics, Ultronique, QR500, 20 kHz, 500W, Brazil). The above extraction protocol was developed for *S. neei* tissues in a study (Souza et al., n.d.) that also showed a high content of free phenolic compounds (FPC) in shoots of this species. Due to the lack of information on phenolic compounds, a larger biomass of the other halophytes was used for extraction. Thus, phenolic compounds in *A. graveolens*, *M. parvifolia*, *P. vaginatum*, *S. terebinthifolius* and *V. luteola* were extracted from 500 mg of mass. Each extract was clarified with 2.5 mL of barium hydroxide 0.1 M and 2.5 mL of zinc sulphate 5%, centrifuged (Centrifuge Eppendorf 5804 R, Germany) at 3220 x *g* and filtrated. The final volume was 25 mL.

2.4. Determination of phenolic acids and quercetin

The identification and quantification of phenolic acids and quercetin in the extracts were carried out by HPLC-UV-Vis (Shimadzu LC-10), equipped with a Discovery Bio Wide Pore C18 column (5 µm, 25 cm x 4.6 mm) (adapted of the Scaglioni, de Souza, Schmidt, & Badiale-Furlong, 2014). The extract was mixed with mobile phase (1:1) and centrifuged at 1400 *g* (Eppendorf Centrifuge 5804 R, Hamburg, Germany) prior to injection. The HPLC-UV-Vis operated at a flow rate of 0.7 mL min^{-1}, at 35 ºC, using a gradient mobile phase consisting of methanol and acidified ultrapure water (glacial acetic acid 1%) (0 min = 30%; 5 min = 40%; 10 min = 50%; 15 min = 60%, and 20 min = 30% methanol; v/v) for 30 min. The wavelength utilized was 280 nm, changing to 320 nm between 14 and 25 min.

2.4.1. Analytical parameters

Chromatographic conditions were validated by their limits of detection and quantification, linearity, efficiency and selectivity (Huber, 2007). A mixture of standard solutions of phenolic acids and quercetin were injected at decreasing concentrations whereas the peak with a ratio of 3:1 times the area of the noise was the limit of detection (LD) (AOAC, 2002). The limit of quantification (LQ) was established as three times the LD (Scaglioni et al., 2014).

Linearity was evaluated by analytical curve, estimated by preparing standard solutions dissolved in the mobile phase and injected into the chromatographic system in triplicate, ranging from LQ value to 21 μg mL^{-1} for each compound. Regression linear was built, curve parameters estimated (e.g. slope, intercept, determination coefficient – R^2), and model assumption (analytical curve) (Ribani, Bottoli, Collins, Jardim, & Melo, 2004). The linearity was calculated by Ribani et al. (2004), consider 10 per cent of variation. After evaluating the normality of the residues by Shapiro-Wilk test, the curve calibration was validated by Analysis of Variance (ANOVA).

The efficiency of separation of the compounds was evaluated by retention (k) and separation (α) calculated according (Meyer, 2004).

2.5. Evaluation of antioxidant activity

The antioxidant activity of Brazilian halophytes was investigated against DPPH radical and peroxidase enzyme (PO) activity. DPPH radical is widely used in the antioxidant capacity evaluation (in 2017 were published more than nineteen thousand papers using this radical, for example) because it is a stable free radical molecule. PO (EC 1.11.1.X) are oxidoreductase enzymes that use a hydrogen peroxide (or other peroxide) to oxidise numerous substances, playing important roles in signalization, lignification process and oxidative defence, processes which may lead to changes in colour and flavour of plants or their fruits (Cruz, Godinho, Aslan, Koçak, & Vieira, 2016; Halliwell & Gutteridge, 2007; Hamid & Khalil-ur-Rehman, 2009). Thus, oxidoreductase capacity, the PO activity can be used to evaluating the antioxidant (enzyme activity inhibition) or prooxidant (enzyme activity favoring) activity.

2.5.1. Determination of DPPH radical scavenging activity

The free-radical scavenging capacity of each extract was adapted from Nicklisch & Waite (2014). The extracts were diluted in three different levels (100, 75 and 50% of extract diluted with extractor solvent), resulting in concentrations of 19, 14 and 10 mg of plant mL^{-1}. Due to lower mass used in extraction, *S. neei* extract present concentrations of 10, 7 and 5 mg plant mL^{-1}. 10 μL of ethanolic DPPH solution (0.80

mg mL^{-1}) were added to 190 µL of extract. Absorbance was measured at 490 nm (TP Reader NM Thermoplate) at intervals of 10 min until 1 h. Measurements started immediately after mixing the solutions. The experiments were performed in triplicate and results express as DPPH inhibition percentage ($\%I_{DPPH}$), estimated according Equation 1:

$$\%I_{DPPH} = [(Abs_{Control} - Abs_{Sample}) / Abs_{Control}] * 100 \quad (1)$$

where $Abs_{control}$ is the absorbance of the solution containing distilled water instead of the sample extracts ($Abs_{control}$ = $Abs_{control}$ with DPPH - $Abs_{control}$ no DPPH); and Abs_{Sample} is the sample extract absorbance (Abs_{Sample} = Abs_{Sample} with DPPH - Abs_{Sample} no DPPH).

In this test, most of plant extracts presented increasing antioxidant activity with extracts' dilution, differently than expected. To better assess the extension of this phenomenon, a second free-radical scavenging analysis was performed with lower concentrations of plant extracts. For the second test, new extractions were performed and extracts of each halophyte were diluted for concentrations of 1, 3 and 5 mg of plant mL^{-1}. DPPH radical scavenging activity was determined according to the above mentioned methodology and results are presented in Figure 2.

2.5.2. Inhibition of peroxidase enzyme (PO) activity

The PO enzyme was obtained from potato (*Solanum tuberosum* L.). Potato and NaCl 0.9 percent aqueous solution was mixed (1:10; w/v) and grinded in knives mill. The extract was twice centrifuged at 3214 *g* (Eppendorf Centrifuge 5804 R, Hamburg, Germany) for 10 min at 4 °C and filtered. Between centrifugation was added 30 ml of acetone, and after centrifugation, the precipitate was suspended in 10 ml NaCl 0.9% into a 25 mL volumetric flask. The protein was quantified (Lowry, Rosebrough, Farr, & Randall, 1951) and a standard curve of bovine serum albumin solubilized in water (18 to 180 µg mL^{-1}) was used to protein estimation in the enzymatic extract.

The PO activity was measured according Schmidt, Gonçalves, Prietto, Hackbart, & Furlong (2014) adapted. Initially, 0.05 mL of phosphate buffer (pH 6.5), distilled water, sample extract, guaiacol solution (1%) and hydrogen peroxide (0.08%) was mixed. After resting for 10 min, 0.05 mL of PO enzymatic extract was added. The

absorbance was read at 490 nm (TP Reader NM Thermoplate) after 20 min, at intervals of 10 min until 1 hour. The experiments were performed in triplicate and results express as PO activity inhibition percentage ($\%I_{PO}$), estimated according Equations 2 and 3:

$$Abs_{Control\ or\ Sample} = (Abs_{tx} - Abs_{0}) / [protein] \quad (2)$$

where Abs_{tx} is the absorbance in different times ($Abs_{tx} = Abs_{Sample\ or\ Control} - Abs_{Enzyme}$); Abs_{t0} is the absorbance initial, time 0 ($Ab_{t0} = Ab_{Sample\ or\ Control} - Ab_{Enzyme}$); and [protein] is the concentration of protein in µg mL^{-1}. And

$$\%I_{PO} = [(Abs_{Control} - Abs_{Sample}) / Abs_{Control}] * 100 \quad (3)$$

where $Abs_{control}$ is the absorbance of the solution containing distilled water; and Abs_{Sample} is the sample extract absorbance.

2.7. Statistical

All determinations were carried out in triplicate and the results obtained were expressed as means and standard deviation.

The concentration of phenolic compounds in the tissues of different halophytes did not agree with the assumptions of normality and homoscedasticity (Kolmogorov-Smirnov and Bartlett tests). Therefore non-parametric tests were applied. Kruskal-Wallis tests with posterior multiple comparisons with Bonferroni correction were used to evaluating differences on phenolic acid and quercetin tissue concentrations among species. Spearman's coefficient of correlation was calculated among FPC (data from the same plant sample published by Souza et al., *in press*) and tissue contents of phenolic acids and quercetin, and antioxidant activities of halophytes' extracts in order to highlight main relationships. In both cited analysis were considered $p < 0.05$.

The contents of phenolic acids in the leaf/shoot tissues of the six halophytes were used as attributes to classify the phenolic acids in a hierarchical clustering procedure. A Pearson correlation algorithm was used to calculate similarity indexes between all pairs of phenolic acids. The WPGMA method (Weighted Pair Group Method with Arithmetic Mean) has been chosen for clustering procedure.

The intensity antioxidant activities (DPPH inhibition percentage and PO activity inhibition percentage) among extracts' dilutions and along the reaction time were evaluated by two-way repeated measures ANOVA. In these ANOVAs repeated factor (within-subject) was the time of consecutive measurement of the antioxidant activities, and the fixed factor (between-subject) was the extract concentration. Greenhouse-Geiseer's corrections were utilized when data violated Mauchly's sphericity test. Arcsin transformation of inhibition percentage values of antioxidant tests {sqrt [arcsin (%I / 100) * (180 / π)]} was applied. As the antioxidant activity of some plants did not fit in the assumptions of normality and homoscedasticity, and more strict ($p < 0.025$) evaluations of ANOVAs were performed (Underwood, 1997). For each halophyte, the relationships between maximum antioxidant activity (independently of extract concentration) and concentrations of phenolic acids and quercetin were evaluated by Spearman correlations. All statistics analysis in this paper was performed in R software (R Core Team, 2018).

3. Results and discussion

3.1 Phenolic compounds

The efficiency of chromatographic method to determine phenolic acids (caffeic, chlorogenic, *p*-coumaric, ferulic, gallic, *p*-hydroxybenzoic, protocatechuic, syringic and vanillin) and the flavonoid quercetin was evaluated by retention (k) and separation (α) factors (see Table 1). In general, the chromatography method presented a good efficiency. The retention factor that shows the interaction of compound between mobile phase and column, ranged between 0.29 and 4.98. The separation factor that measures of separation of adjacent compounds ranged between 1.05 and 2.74. All compounds presents good values of k ($1 < k < 10$) and α ($\alpha > 1$) (Meyer, 2004). In addition, all analytical curves presented correlation coefficients (R^2) higher than 0.99 ($p = 0.001$).

As shown in Table 1, the LD and LQ values ranged from 2.00 to 25.0 ng $mL^{-1}$and 5.00 to 75.0 ng mL^{-1}, respectively. These results show that the chromatography method developed was more sensitive than applied in extracts of *Oryza sativa* bran (HPLC-UV-Vis, column C18 and 10 µm particle size) (Scaglioni et al., 2014), and of *S. neei* shoots (HPLC-ESI-MS/MS, column C8 and 3.5 µm particle size) (Bertin et al.,

2014), which obtained average LD of 248 and 69 ng mL^{-1}, and average LQ of 743 and 243 ng mL^{-1}, respectively.

In general, the results revealed the presence of nine phenolic acids, present in all species. This work was the first to evaluate the phenolic acids (caffeic, chlorogenic, p-coumaric, ferulic, gallic, p-hydroxybenzoic, protocatechuic, syringic and vanillin) content in leaves of *M. parvifolia*, *P. vaginatum* and *V. luteola*. The sum of phenolic acids' contents showed a ten-fold difference among halophytes species (45.2 to 453.0 µg g^{-1} dw; dry weight) and these values were higher than mean concentrations found in pineapple (*Ananas comosus*, 37.4 µg g^{-1}dw) (Bataglion, Da Silva, Eberlin, & Koolen, 2015), garlic (*Allium sativium*, 24.6 µg g^{-1} dw) and spinach (*Spinacia oleracea*, 33.6 µg g^{-1} dw) (Alarcón-Flores, Romero-González, Vidal, & Frenich, 2014). Except by *S. neei*, halophytes showed a sum of phenolic acid higher than acerola (*Malpighia emarginata*, 49.6 µg g^{-1} dw) (Bataglion et al., 2015), celery root (*Apium graveolens* var. rapaceum: 90.2 µg g^{-1} dw) and red onion (*Allium cepa*: 73.8 µg g^{-1} dw) (Mattila & Hellström, 2007). The salt marsh variety of *A. graveolens* presents the highest leaf sum of phenolic acids among halophytes, and it also surpassed the amount found in commercial celery (var. rapaceum) (Mattila & Hellström, 2007), artichoke (*Cynara scolymus*:142 µg g^{-1} dw) (Bataglion et al., 2015) and Açaí-do-Amazonas fruit (*Euterpe precatoria*: 178 µg g^{-1} dw) (Alarcón-Flores et al., 2014). On the other hand, the total amount of phenolic acids found represent a very small fraction of FPC concentration in halophytes' tissue, whose values were quantified in the same tissue samples by Souza et al. (*in press*). Even for *A. graveolens*, the sum of phenolic acids content corresponds to only 2.8% of FPC. This later result is not unexpected since we analyzed only 8.3% of all existing phenolic acids (9 of 108) listed by Rothwell et al. (2013).

The quercetin content pattern among halophytes (*S. terebinthifolius* > *V. luteola* > *S. neei*; and not detected in *A. graveolens, M. parvifolia and P. vaginatum*) was different from the observed total content of phenolic acids (Table 2). This study is the first to report the quercetin content in *V. luteola* and the concentration of this flavonoid in leaves and shoots of the three above cited halophytes were higher than spinach (1.90 µg g^{-1} dw), artichoke (1.70 µg g^{-1} dw) and pineapple (0.76 µg g^{-1} dw), but lower than fruits of Açaí-do-Amazonas (136 µg g^{-1} dw) and acerola (27.7 µg g^{-1} dw) (Alarcón-Flores et al., 2014; Bataglion et al., 2015; Mattila & Hellström, 2007).

The profiles of phenolic acids of the selected halophytes were quite variable (Table 2). *Apium graveolens* showed significantly highest individual concentrations of three phenolic acids (chlorogenic, *p*-hydroxybenzoic and caffeic acid). It is highlighting that *A. graveolens* leaves had an average 295 µg g^{-1} dw of chlorogenic acid, and the content of this ester of caffeic and quinic acids was 50-1000% higher than values found in other varieties of celery (85.7 – 119 µg g^{-1} dw) (Sorour, Hassanen, & Ahmed, 2015). Further, the chlorogenic acid content in *A. gravelens* was higher than spinach (3.12 µg g^{-1} dw), Açaí-do-amazonas fruit (9.09 µg g^{-1} dw) and artichoke (131 µg g^{-1} dw) (Alarcón-Flores et al., 2014; Bataglion et al., 2015; Mattila & Hellström, 2007). The average levels of *p*-coumaric acid in *A. graveolens*, *M. parvifolia* and *S. terebinthifolius* were also higher than found in Açaí-do-Amazonas fruit (1.22 µg g^{-1} dw) (Bataglion et al., 2015). Coumaric acid is one of the main responsible for the antioxidant activity of plants, as well as for caffeic acid (Goleniowski et al., 2013). A major concentration of ferulic acid was detected in *S. terebinthifolius*. The content of caffeic and ferulic acids in shoots and leaves of studied halophytes were equal or higher than pineapple, avocado (*Persea americana*), acerola and Açaí-do-Amazonas fruits, garlic, red onion, artichoke, and spinach, but lower than tomato (*Lycopersicon esculentum*), broccoli (*Brassica oleracea* var. italica), and red cabbage (*Brassica oleracea* var. capitata "f. rubra") (Alarcón-Flores et al., 2014; Bataglion et al., 2015). Except *S. neei* and *V. luteola*, the halophyte species present high contents of protocatechuic acid, higher than broccolli (11.1 µg g^{-1}dw) (Alarcón-Flores et al., 2014) and Açaí-do-Amazonas fruit (7.17 µg g^{-1}dw) (Bataglion et al., 2015). The *p*-hydroxybenzoic and syringic acids concentration were similar those found in fruits and vegetables (e.g. broccoli, red cabbage and acerola) with proven bioactivity (e.g. antioxidant activity). Vanillin concentration ranged between 1.30 (*V. luteola*) to 6.13 µg g^{-1} dw (*S. neei*), and it is a phenolic aldehyde little studied and a primary component of the extract of vanilla beans (Dong, Gu, Xu, & Wang, 2014). Gallic acid is a powerful biomolecule antioxidant, anti-inflammatory and anticancer (and others bioactivity) (Goleniowski et al., 2013), and it's content in *P. vaginatum* and *V. luteola* was below than limit of quantification, but averages 23.2 µg g^{-1} dw for *S. terebinthifolius* leaves were achieved. This later value was higher than found in Açaí-do-Amazonas and acerola fruits (Bataglion et al., 2015) but smaller those found in rice (22.7 – 44.6 µg g^{-1}dw) (Scaglioni et al., 2014).

Phenolic acids profile can be diverse, especially in polyphyletic groups such as halophytes. Indeed, the most high concentration of each phenolic acid was observed in a different halophyte species (Table 2; *A. graveolens*- chlorogenic; *M. parvifolia*- ferulic; *P. vaginatum*- protocatechuic; *S. neei*- caffeic; *S. terebinthifolius*- ferulic; and *V. luteola*- syringic). Cluster analysis identified three distinct groups of phenolic acids with negative weighted average intergroup correlations (Figure 1). Group I included the acids caffeic, chlorogenic, *p*-hydroxybenzoic, protocatechuic and vanillin, all found in the most salt tolerance halophytes studied (Table 2), which inhabit more frequently flooded tidal levels and/or áreas subjected to very high salt concentrations in soil (i.e., *S. neei*, *A. graveolens* and *P. vaginatum*) (Costa & Herrera, 2016). High content of chlorogenic and caffeic acids were found also in others halophytes, such as *Artemisia scoparia* and *Plantago major* (Ksouri et al., 2012). Glycophytes species such sage (*Salvia officinalis*) (Bettaieb, Hamrouni-Sellami, Bourgou, Limam, & Marzouk, 2011) and artichoke (Rezazadeh, Ghasemnezh, Barani, & Telmadarre, 2012), showed increasing leaf content of these two acids with the increment of salt stress. Furthermore, high synthesis of chlorogenic acid in transgenic than in not modified tomato (*L. esculentum* var Moneymaker) results in better protection against oxidative stress (Niggeweg, Michael, & Martin, 2004).

The group II of phenolic acids was composed by ferulic, gallic and *p*-coumaric acids, all very abundant in leaves of *M. parvifolia* and *S. terebinthifolius*, species that frequently inhabit the upper salt marshes and sandy plains throughout the Brazilian coast (Baggio, 1988; Freitas & Kinoshita, 2015). Ferulic and *p*-coumaric acid are precursors of lignin (Goleniowski et al., 2013) and the positive correlation of this two acids may be partially related to their mutual role the lignin biosynthesis. Leaves of *S. terebinthifolius* (Arato et al., 2017) and *M. salicina* (same genus of *M. parvifolia*) are rich in lignin, around 20% of dry weight (Wardle, Bonner, & Barker, 2002). The group III of Cluster Analysis was composed by syringic acid, whose halophytes´ concentrations lack correlation with other phenolic acids. Syringic acid is widely present compound in species of *Fabaceae* family (Wink, 2013) as it was found for the marsh beans *V. luteola*.

3.2. Antioxidant activities

In general, the ethanolic extracts of all halophytes show high DPPH radical scavenging activity (%I_{DPPH} > 80.0%) (Table 3) that surpasses broccoli (*Brassica oleracea* var. botrytis, 10 mg dw mL^{-1}), green bean (*Phaseolus vulgaris*, 10 mg dw mL^{-1}), and tomato (10 mg dw mL^{-1}) (Aires, Carvalho, & Saavedra, 2017). *Salicornia neei* extract showed the highest antioxidant activity (99.3 %I_{DPPH}), followed by *S. terebinthifolius*, *M. parvifolia*, *V. luteola*, *P. vaginatum* and *A. graveolens*. The maximum DPPH inhibition was positively correlation with quercetin (R^2 = 0.67) and ferulic acid (R^2 = 0.66) content (Spearman's correlation), compounds with high antioxidant capacity (Ghasemzadeh & Ghasemzadeh, 2011; Yeh & Yen, 2003), and negatively with benzoic acids (protocatechuic (R^2) = -0.82; and *p*-hydroxybenzoic (R^2) = -0.59).

The extracts of Brazilian halophytes showed different reaction times to reach their maximum inhibition of scavenger radicals and they can be listed in order of fastest to slowest antioxidant capacity (higher %I_{DPPH} in 10 minutes): *S. terebinthifolius*, *M. parvifolia*, *P. vaginatum*, *A. graveolens*, *V. luteola* and *S. neei*. Although showing highest final antioxidant capacity, DPPH inhibition by *S. neei* extracts slowly increase with reaction time (Time (T); F= 621, $p < 0.025$) as well as *V. luteola* (F= 179, $p < 0.025$). Slowly DPPH radical scavenging of *S. neei* extract can be associated with its more diluted extract concentration. The %I_{DPPH} velocity of *V. luteola* extracts may be associated with the high content of syringic acid. Syringic acid is a cinnamic acid with low antioxidant capacity (Yeh & Yen, 2003) and represents around 50 percent of *V. luteola* phenolic acid content. *Salicornia neei* and *V. luteola* presented significant interaction between extract concentration (C) and T (F= 45.0, $p < 0.025$) showed that antioxidant activity get slower with decreasing extract concentrations.

Myrsine parvifolia (F= 36.6, $p < 0.025$) and *S. neei* (F= 397, $p < 0.025$) showed significant relationships between concentration of extract and DPPH radical scavenging activity (Table 3). Their highest antioxidant activities were not observed in the most concentrated extracts but in diluted ones (concentrations of 10 and 7 mg of plant mL^{-1} for *M. parvifolia* and *S. neei*, respectively). Except for *A. graveolens,* all other species showed higher average values (although not significances) at diluted concentrations (Table 3). Overall dilution appeared to favour the antioxidant performance of these species. This hypothesis was evaluated by antioxidant test with more diluted extracts (second experiment). Quadratic function responses of the antioxidant activity with in-

creasing concentration of halophytes' extracts were obtained (Figure 2). Thus, for all species, maximum antioxidant activity was observed in a specific dilution of the plant extract and further dilution lead to decreasing capacity to refrain radical scavenging activity. *Apium graveolens* and *M. parvifolia* results demonstrated a more abrupt drop of $\%I_{DPPH}$ with the increase of extract concentration than other halophytes.

Similar responses were observed in quite different extracts' composition. This quadratic response behaviour of antioxidant activity to extract concentrations is probably associated with matrix characteristics (e.g. pH and metals contents), which in addition with the concentration, solubility and bioavailable of individual metabolites, may influence the antioxidant capacity of the phenolic compounds (Halliwell & Gutteridge, 2007; Quideau et al., 2011). For example, gallic acid is quickly oxidized in pH higher than 7, losing its antioxidant capacity (Strlič, Radovič, Kolar, & Pihlar, 2002). In addition, plants halophytes have high capacity of sequester from soil metal ions (Flowers & Colmer, 2015), and countless of these elements (e.g. As, Cu, Fe, Ni and Zn) can catalyse prooxidant activity of phenolic acids, quercetin and vitamins C and E (Eghbaliferiz & Iranshahi, 2016; Strlič et al., 2002). Summing up, extract dilution can favours $\%I_{DPPH}$ by reducing interferences from other matrix components. However, in elevated dilutions, the concentration of antioxidant compounds decrease significantly, resulting in reduction of antioxidant activity. Further studies of antioxidant activity with controlled halophyte matrices (i.e., plants cultivated in different metal concentrations) are needed in order to confirm this hypothesis.

Concerning the evaluation of antioxidant activity by the inhibition of PO activity, *S. terebinthifolius* and *M. parvifolia* (group II of phenolic acids) showed high $\%I_{PO}$, with 98.6 and 87.6% of maximum inhibition, respectively, followed in decreasing order of $\%I_{PO}$ by *S. neei*, *P. vaginatum, A. graveolens* (all three species in group I of phenolic acids) and *V. luteola* (Table 4). The level of PO inhibition of halophytes ethanolic extracts was higher than observed for rice bran, found around 0.1 mg mL^{-1} of rice bran extract attain 60 % of PO inhibition (Schmidt et al., 2014), and similar the found in *Gentiana lutea* extracts (0.01 mg mL^{-1}) but with different solvents (Nastasijević et al., 2012). Negative $\%I_{PO}$ values were observed in some halophytes species (Table 4), which indicate prooxidant behaviour. Prooxidant activity was found in *V. luteola* (all extract concentration), *S. neei* (all extract concentration), *M. parvifolia* (8 mg mL^{-1}), and *P. va-*

ginatum (4 mg mL^{-1}). Since PO have important roles in plants, as removal hydrogen peroxide, deposition of lignin and defence against of pathogen (Halliwell & Gutteridge, 2007; Hamid & Khalil-ur-Rehman, 2009), the bioactive property of the extracts favouring PO activity (*i.e.* negative values of $\%I_{PO}$) can be an important feature of plant homoeostasis. As for the $\%I_{DPPH}$, no general pattern $\%I_{PO}$ over different extract concentrations and reactions time was found for all species (Table 4). The extract concentration of *M. parvifolia* ($F= 18.2$, $p < 0.025$), *S. neei* ($F= 28.1$, $p < 0.025$) and *V. luteola* ($F= 45.9$, $p < 0.025$) affects significantly $\%I_{PO}$. *S. neei* extracts became more prooxidants with decreasing concentrations, whereas *M. parvifolia* extracts showed significantly lower $\%I_{PO}$ values at intermediated dilution (8 mg mL^{-1}). Only *V. luteola*, which has a very distinct phenolic acids profile (group III of phenolic acids), showed a significant increase of the antioxidant activity over time ($F= 76.6$, $p < 0.025$), particularly observed at its lowest extract concentration (significant interaction C x T; $F= 24.9$).

Inhibition of PO activity was significantly correlated with only caffeic acid ($R^2 = -0.64$), a phenolic acid with high prooxidant activity (Eghbaliferiz & Iranshahi, 2016). However, the variability of the PO activity responses between species seems associated with the distinct groups of phenolic acids profiles observed in our study. Halophyte is polyphyletic group, with multiple origins, but to survive in the same coastal habitat they must have convergent biochemical mechanisms to withstand salt stress, periodical flooding or even prolonged droughts (Rozema & Schat, 2013), what may lead to similar chemical proportionality (ratios) instead similar concentrations of compounds. On the other hand, phenolic compounds profiles in plants are varied and diversified, encompassing more than eight thousand compounds of phenolic acids, flavonoids, lignin, others (Quideau et al., 2011; Rothwell et al., 2013), and several of those were not included by the present study. Furthermore, different compounds rather than phenolic acids and quercetin are present in the species extracts analysed, along with various peroxide forms that, for instance, could be reduced by guaiacol oxidation.

4. Conclusions

This paper was the first to show phenolic acids and quercetin content in foliar tissues of *Myrsine parvifolia*, *Paspalum vaginatum* and *Vigna luteola*. The Brazilian

halophytes showed high and diversified composition of phenolic acids and quercetin. In general, all species present high antioxidant capacity, but particularly *S. terebinthifolius* and *M. parvifolia*, which had higher and faster antioxidant response against both free radical tested (DPPH and H_2O_2) and similarly high contents of ferulic and gallic acids in their tissues. Plant extract concentration showed a quadratic relationship with DPPH radical antioxidant activity and the individual contents of phenolic compounds were not correlated with antioxidant activities. These two last observations may be explained by an effect of the plant matrix, possibly because of the presence of metals and metalloids in halophytes` tissues and their interference on antioxidant capacity of metabolites. Our results highlight the importance of conducting preliminary tests for optimisation of chemical analyses with groups of species that are still little studied. Pro-oxidant activity of halophyte extracts was detected by PO enzyme inhibition. As for antioxidant capacity, pro-oxidant has numerous benefits effects to plant and human health.

5. References

Aires, A., Carvalho, R., & Saavedra, M. J. (2017). Reuse potential of vegetable wastes (broccoli, green bean and tomato) for the recovery of antioxidant phenolic acids and flavonoids. *International Journal of Food Science & Technology*, *52*(1), 98–107. https://doi.org/10.1111/ijfs.13256

Alarcón-Flores, M. I., Romero-González, R., Vidal, J. L. M., & Frenich, A. G. (2014). Determination of phenolic compounds in artichoke, garlic and spinach by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry. *Food Analytical Methods*, *7*(10), 2095–2106. https://doi.org/10.1007/s12161-014-9852-4

Amirul Alam, M., Juraimi, a. S., Rafii, M. Y., Hamid, a. a., Aslani, F., & Alam, M. Z. (2015). Effects of salinity and salinity-induced augmented bioactive compounds in purslane (*Portulaca oleracea* L.) for possible economical use. *Food Chemistry*, *169*, 439–447. https://doi.org/10.1016/j.foodchem.2014.08.019

AOAC. (2002). *Validation of chemical methods for dietary. Supplements and botanicals* (Guidelines for single laboratory). *AOAC International*.

Arato, H. D., Dias, L. E., Assis, I. R. de, Barros, N. F. de, Neves, J. C. L., & Schaefe, C. E. G. R. (2017). Leaf residue decomposition of selected Atlantic forest tree

species. *Revista Árvore*, *41*(3), 1–9. https://doi.org/http://dx.doi.org/10.1590/1806-90882017000300020

Baggio, A. J. (1988). Aroeira como potencial para usos múltiplos na propriedade rural. *Boletim de Pesquisa Florestal*, *17*, 25–32.

Bataglion, G. A., Da Silva, F. M. A., Eberlin, M. N., & Koolen, H. H. F. (2015). Determination of the phenolic composition from Brazilian tropical fruits by UHPLC-MS/MS. *Food Chemistry*, *180*, 280–287. https://doi.org/10.1016/j.foodchem.2015.02.059

Bertin, R. L., Gonzaga, L. V., Borges, G. da S. C., Azevedo, Mô. S., Maltez, H. F., Heller, M., … Fett, R. (2014). Nutrient composition and, identification/quantification of major phenolic compounds in *Sarcocornia ambigua* (Amaranthaceae) using HPLC-ESI-MS/MS. *Food Research International*, *55*, 404–411. https://doi.org/10.1016/j.foodres.2013.11.036

Bettaieb, I., Hamrouni-Sellami, I., Bourgou, S., Limam, F., & Marzouk, B. (2011). Drought effects on polyphenol composition and antioxidant activities in aerial parts of *Salvia officinalis* L. *Acta Physiologiae Plantarum*, *33*(4), 1103–1111. https://doi.org/10.1007/s11738-010-0638-z

Boxall, A. B. A., Rudd, M. A., Brooks, B. W., Caldwell, D. J., Choi, K., Hickmann, S., … Van Der Kraak, G. (2012). Pharmaceuticals and personal care products in the environment: What are the big questions? *Environment Health Perspectives*, *120*(9), 1221–1229. https://doi.org/http://dx.doi.org/10.1016/j.envint.2013.06.012

Costa, C. S. B., & Herrera, O. B. (2016). Halophytic Life in Brazilian Salt Flats: Biodiversity, Uses and Threats. In M. A. Khan, B. Boër, M. Ozturk, M. Clüsener-Godt, B. Gul, & S.-W. Breckle (Eds.), *Sabkha Ecosystem V : The Americas* (1ª, pp. 11–27). Berlin: Springer International Publishing. https://doi.org/10.1007/978-3-319-27093-7_2

Costa, C. S. B., Vicenti, J. R. M., Morón-Villareyes, J. A., Caldas, S., Cardoso, L. V, Freitas, R. F., & D'Oca, M. G. M. (2014). Extraction and characterization of lipids from *Sarcocornia ambigua* meal: a halophyte biomass produced with shrimp farm

effluent irrigation. *Anais Da Academia Brasileira de Ciências*, *86*(2), 935–943. https://doi.org/10.1590/0001-3765201420130022

Cotrim, G., Fahning, C. S., Rocha, G. O. da, & Hatje, V. (2016). Endocrine disruptors: strategies for determination and occurrence in marine environments. *Revista de Gestão Costeira Integrada*, *16*(3), 299–326. https://doi.org/10.5894/rgci669

Crozier, A., Jaganath, I. B., & Clifford, M. N. (2009). Dietary phenolics: chemistry, bioavailability and effects on health. *Natural Product Reports*, *26*(8), 1001–1043. https://doi.org/10.1039/b802662a

Cruz, R. M. S., Godinho, A. I. A., Aslan, D., Koçak, N. F., & Vieira, M. C. (2016). Modeling the kinetics of peroxidase inactivation, colour and texture changes of Portuguese cabbage (*Brassica oleracea* L. var. costata DC) during UV-C light and heat blanching. *International Journal of Food Studies*, *5*(2), 180–192. https://doi.org/10.7455/ijfs/5.2.2016.a6

Dong, Z., Gu, F., Xu, F., & Wang, Q. (2014). Comparison of four kinds of extraction techniques and kinetics of microwave-assisted extraction of vanillin from Vanilla planifolia Andrews. *Food Chemistry*, *149*, 54–61. https://doi.org/10.1016/j.foodchem.2013.10.052

Eghbaliferiz, S., & Iranshahi, M. (2016). Prooxidant activity of polyphenols, flavonoids, anthocyanins and carotenoids: Updated review of mechanisms and catalyzing metals. *Phytotherapy Research*, (March), 1379–1391. https://doi.org/10.1002/ptr.5643

Flowers, T. J., & Colmer, T. D. (2015). Plant salt tolerance: adaptations in halophytes. *Annals of Botany*, *115*(3), 327–331. https://doi.org/10.1093/aob/mcu267

Freitas, M. de F., & Kinoshita, L. S. (2015). Myrsine (Myrsinoideae-Primulaceae) no sudeste e sul do Brasil. *Rodriguesia*, *66*(1), 167–189. https://doi.org/10.1590/2175-7860201566109

Ghasemzadeh, A., & Ghasemzadeh, N. (2011). Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *Journal of Medicinal Plants Research*, *5*(31), 6697–6703. https://doi.org/10.5897/JMPR11.363

Giada, M. (2013). Food Phenolic Compounds: Main Classes, Sources and Their Antioxidant Power. In J. A. Morales-González (Ed.), *Oxidative Stress and Chronic Degenerative Diseases - A Role for Antioxidants* (pp. 87–112). https://doi.org/10.5772/51687

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, *48*(12), 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Goleniowski, M., Bonfill, M., Cusido, R., & Palazón, J. (2013). Phenolic Acids. In *Natural Products* (pp. 1951–1973). Berlin, Heidelberg: Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-22144-6_64

Halliwell, B., & Gutteridge, J. M. C. (2007). *Free radicals in biology and medicine* (Fourth). New York, USA: Oxford University Press.

Hamid, M., & Khalil-ur-Rehman. (2009). Potential applications of peroxidases. *Food Chemistry*, *115*(4), 1177–1186. https://doi.org/10.1016/j.foodchem.2009.02.035

Heleno, S. A., Martins, A., Queiroz, M. J. R. P., & Ferreira, I. C. F. R. (2015). Bioactivity of phenolic acids: Metabolites versus parent compounds: A review. *Food Chemistry*, *173*, 501–513. https://doi.org/10.1016/j.foodchem.2014.10.057

Huber, L. (2007). *Validation and qualification in analytical laboratories* (2ª). New York: CRC Press.

Jithesh, M. N., Prashanth, S. R., Sivaprakash, K. R., & Parida, A. K. (2006). Antioxidative response mechanisms in halophytes: Their role in stress defence. *Journal of Genetics*, *85*(3), 237–254. https://doi.org/10.1007/BF02935340

Joven, J., Micol, V., Segura-Carretero, A., Alonso-Villaverde, C., Menéndez, J. A., Aragonès, G., … Vázquez-Martín, A. (2014). Polyphenols and the Modulation of Gene Expression Pathways: Can We Eat Our Way Out of the Danger of Chronic Disease? *Critical Reviews in Food Science and Nutrition*, *54*(8), 985–1001. https://doi.org/10.1080/10408398.2011.621772

Ksouri, R., Ksouri, W. M., Jallali, I., Debez, A., Magné, C., Hiroko, I., & Abdelly, C. (2012). Medicinal halophytes: Potent source of health promoting

biomolecules with medical, nutraceutical and food applications. *Critical Reviews in Biotechnology*, *32*(4), 289–326. https://doi.org/10.3109/07388551.2011.630647

Lattanzio, V., Cardinali, A., Ruta, C., Fortunato, I. M., Lattanzio, V. M. T., Linsalata, V., & Cicco, N. (2009). Relationship of secondary metabolism to growth in oregano (*Origanum vulgare* L.) shoot cultures under nutritional stress. *Environmental and Experimental Botany*, *65*(1), 54–62. https://doi.org/10.1016/j.envexpbot.2008.09.002

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*, *193*(1), 265–275.

Mattila, P., & Hellström, J. (2007). Phenolic acids in potatoes, vegetables, and some of their products. *Journal of Food Composition and Analysis*, *20*(3–4), 152–160. https://doi.org/10.1016/j.jfca.2006.05.007

Meyer, V. R. (2004). *Practical High-Performance Liquid Chromatography* (Four editi). John Wiley & Sons.

Miller, G., Suzuki, N., Ciftci-Yilmaz, S., & Mittler, R. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, Cell and Environment*, *33*(4), 453–467. https://doi.org/10.1111/j.1365-3040.2009.02041.x

Nastasijević, B., Lazarević-Pašti, T., Dimitrijević-Branković, S., Pašti, I., Vujačić, A., Joksić, G., & Vasić, V. (2012). Inhibition of myeloperoxidase and antioxidative activity of *Gentiana lutea* extracts. *Journal of Pharmaceutical and Biomedical Analysis*, *66*, 191–196. https://doi.org/10.1016/j.jpba.2012.03.052

Nicklisch, S. C. T., & Waite, J. H. (2014). Optimized DPPH assay in a detergent-based buffer system for measuring antioxidant activity of proteins. *MethodsX*, *1*, 233–238. https://doi.org/10.1016/j.mex.2014.10.004

Niggeweg, R., Michael, A. J., & Martin, C. (2004). Engineering plants with increased levels of the antioxidant chlorogenic acid. *Nature Biotechnology*, *22*(6), 746–754. https://doi.org/10.1038/nbt966

Panta, S., Flowers, T., Lane, P., Doyle, R., Haros, G., & Shabala, S. (2014). Halophyte agriculture: Success stories. *Environmental and Experimental Botany*, *107*, 71–83. https://doi.org/10.1016/j.envexpbot.2014.05.006

Pinheiro, I., Arantes, R., do Espírito Santo, C. M., do Nascimento Vieira, F., Lapa, K. R., Gonzaga, L. V., … Seiffert, W. Q. (2017). Production of the halophyte *Sarcocornia ambigua* and Pacific white shrimp in an aquaponic system with biofloc technology. *Ecological Engineering*, *100*, 261–267. https://doi.org/10.1016/j.ecoleng.2016.12.024

Quideau, S., Deffieux, D., Douat-Casassus, C., & Pouységu, L. (2011). Plant polyphenols: Chemical properties, biological activities, and synthesis. *Angewandte Chemie - International Edition*, *50*(3), 586–621. https://doi.org/10.1002/anie.201000044

R Core Team. (2018). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Rezazadeh, A., Ghasemnezh, A., Barani, M., & Telmadarre, T. (2012). Effect of Salinity on Phenolic Composition and Antioxidant Activity of Artichoke (*Cynara scolymus* L.) Leaves. *Research Journal of Medicinal Plant*, *6*(3), 245–252. https://doi.org/10.3923/rjmp.2012.245.252

Ribani, M., Bottoli, C. B. G., Collins, C. H., Jardim, I. C. S. F., & Melo, L. F. C. (2004). Validaçâo em métodos cromatográficos e eletroforéticos. *Quimica Nova*, *27*(5), 771–780. https://doi.org/10.1590/S0100-40422004000500017

Rothwell, J. A., Perez-Jimenez, J., Neveu, V., Medina-Remon, A., M'Hiri, N., Garcia-Lobato, P., … Scalbert, A. (2013). Phenol-Explorer 3.0: a major update of the Phenol-Explorer database to incorporate data on the effects of food processing on polyphenol content. *Database*, *2013*, 1–8. https://doi.org/10.1093/database/bat070

Rozema, J., & Schat, H. (2013). Salt tolerance of halophytes, research questions reviewed in the perspective of saline agriculture. *Environmental and Experimental Botany*, *92*, 83–95. https://doi.org/10.1016/j.envexpbot.2012.08.004

Salin, M. L. (1988). Toxic oxygen species and protective systems of the chloroplast. *Physiologia Plantarum*, *72*(3), 681–689. https://doi.org/https://doi.org/10.1111/j.1399-3054.1988.tb09182.x

Scaglioni, P. T., de Souza, T. D., Schmidt, C. G., & Badiale-Furlong, E. (2014). Availability of free and bound phenolic compounds in rice after hydrothermal treatment. *Journal of Cereal Science*, *60*(3), 526–532. https://doi.org/10.1016/j.jcs.2014.08.005

Schmidt, C. G., Gonçalves, L. M., Prietto, L., Hackbart, H. S., & Furlong, E. B. (2014). Antioxidant activity and enzyme inhibition of phenolic acids from fermented rice bran with fungus *Rizhopus oryzae*. *Food Chemistry*, *146*, 371–377. https://doi.org/10.1016/j.foodchem.2013.09.101

Shrivastava, A. K., Patra, S., & Tikariha, A. (2016). Uses of weeds as Medicine in Durg District of Chhattisgarh. *Indian Journal of Applied & Pure Biology*, *31*(1), 91–104.

Sorour, M. A., Hassanen, N. H. M., & Ahmed, M. H. M. (2015). Natural Antioxidant Changes in Fresh and Dried celery (*Apium graveolens*). *American Journal of Energy Engineering*, *3*, 12–16. https://doi.org/10.11648/j.ajee.s.2015030201.13

Souza, M. M., da Silva, B., Costa, C. S. B., & Badiale-Furlong, E. (*in press*). Free phenolic compounds extraction from Brazilian halophytes, soybean and rice bran by ultrasound-assisted and orbital shaker methods. *Anais Da Academia Brasileira de Ciencias*.

Sreenivasulu, N., Grimm, B., Wobus, U., & Weschke, W. (2000). Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of foxtail millet (*Setaria italica*). *Physiologia Plantarum*, *109*(4), 435–442. https://doi.org/10.1034/j.1399-3054.2000.100410.x

Strlič, M., Radovič, T., Kolar, J., & Pihlar, B. (2002). Anti- and prooxidative properties of gallic acid in fenton-type systems. *Journal of Agricultural and Food Chemistry*, *50*(22), 6313–6317. https://doi.org/10.1021/jf025636j

Suffredini, I. B., Paciencia, M. L., Varella, A. D., & Younes, R. N. (2006). Antibacterial activity of Brazilian Amazon plant extracts. *The Brazilian Journal of Infectious Diseases*, *10*(6), 400–402. https://doi.org/http://dx.doi.org/10.1590/S1413-86702006000600008

Uddin, K., Juraimi, A. S., Ismail, M. R., Hossain, A., Othman, R., & Abdul Rahim, A. (2012). Physiological and growth responses of six turfgrass species relative

to salinity tolerance. *The Scientific World Journal*, *2012*, Article ID 905468, 10 pages. https://doi.org/10.1100/2012/905468

Uliana, M. P., Fronza, M., da Silva, A. G., Vargas, T. S., de Andrade, T. U., & Scherer, R. (2016). Composition and biological activity of Brazilian rose pepper (*Schinus terebinthifolius* Raddi) leaves. *Industrial Crops and Products*, *83*, 235–240. https://doi.org/10.1016/j.indcrop.2015.11.077

Underwood, A. J. (1997). *Experiments in Ecology. Their logical design and interpretation using analysis of variance*. Cambridge, United Kingdom: Cambridge University Press.

Wardle, D. A., Bonner, K. I., & Barker, G. M. (2002). Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. *Functional Ecology*, *16*, 585–595. https://doi.org/https://doi.org/10.1046/j.1365-2435.2002.00659.x

Wink, M. (2013). Evolution of secondary metabolites in legumes (Fabaceae). *South African Journal of Botany*, *89*, 164–175. https://doi.org/10.1016/j.sajb.2013.06.006

Yao, Y., Sang, W., Zhou, M., & Ren, G. (2010). Phenolic composition and antioxidant activities of 11 celery cultivars. *Journal of Food Science*, *75*(1), C9–C13. https://doi.org/10.1111/j.1750-3841.2009.01392.x

Yeh, C. T., & Yen, G. C. (2003). Effects of phenolic acids on human phenolsulfotransferases in relation to their antioxidant activity. *Journal of Agricultural and Food Chemistry*, 51(5), 1474–1479. https://doi.org/10.1021/jf0208132

Table 1. Analytical curve parameters of the chromatographic method for determination of phenolic acids and quercetin.

N	Analyte	Rt (min)	k	α	LD (ng mL^{-1})	LQ (ng mL^{-1})	Analytical curve	Linearity (µg mL^{-1})	R^2	*P*-value
1	Gallic	5.18	0.29	2.74	12.5	37.5	y= 103396*x+ 1030	0.25 – 3.00	0.999	0.001
2	Protocatechiuc	7.21	0.79	1.73	12.5	37.5	Y = 67433*x + 1003	0.20 – 6.00	1.000	0.001
3	Chlorogenic	9.61	1.37	1.19	5.00	15.0	y = 50931*x + 1571	0.50 – 6.00	0.999	0.001
4	*p*-Hydroxybenzoic	10.6	1.63	1.13	5.00	15.0	y = 68760*x + 1076	0.20 – 6.00	0.999	0.001
5	Caffeic	11.4	1.84	1.05	5.00	15.0	y = 100698*x + 1201	0.25 – 6.00	0.999	0.001
6	Syringic	11.8	1.94	1.13	5.00	15.0	y = 100155*x + 689.1	0.20 – 6.00	0.999	0.001
7	Vanillin	12.8	2.19	1.27	25.0	75.0	y =148473*x – 1008	0.10 – 6.00	0.999	0.001
8	*p*-Coumaric	15.2	2.78	1.05	1.60	4.80	y = 403022*x + 6160	0.25 – 6.00	0.999	0.001
9	Ferulic	15.7	2.92	1.71	1.60	4.80	y = 319304*x + 4508	0.25 – 6.00	0.999	0.001
10	Quercetin	24.0	4.98	-	25.0	75.0	y = 52617*x – 768.1	0.25 – 6.00	0.999	0.001

N – number of phenolic acids and quercetin; R_t – Retention time; k – Retention factor; α – Separation factor; LD – Limit of detection; LQ – Limit of quantification; R^2 – Determination coefficient; p-value – Referent the results of Analysis of Variance (ANOVA) of linear model (regression).

Table 2. Phenolic acids profile, quercetin, and total free phenolic compounds (FPC) in *Apium graveolens*, *Paspalum vaginatum*, *Salicornia neei*, *Schinus terenbithifolius, Myrsine parvifolia and Vigna luteola* (mean ± standard deviation; µg g^{-1} dry weight). The two halophyte species with most high concentrations of each phenolic acids were highlighted in black and gray colours.

	A. graveolens	*P. vaginatum*	*S. neei*	*S. terebinthifolius*	*M. parvifolia*	*V. luteola*
Phenolic acids						
Chlorogenic	295 ± 22.8[a]	15.6 ± 0.05[b]	7.97 ± 0.39[d]	10.5 ± 0.11[c]	3.70 ± 0.13[e]	0.31 ± 0.51[f]
Caffeic	72.8 ± 2.09[a]	10.5 ± 0.21[e]	15.0 ± 0.24[c]	11.4 ± 0.55[d]	< LQ	15.5 ± 0.07[b]
p-Hydroxybenzoic	34.1 ± 2.82[a]	13.9 ± 0.19[b]	6.58 ± 0.25[d]	11.7 ± 1.38[bc]	3.46 ± 0.10[e]	9.93 ± 0.91[c]
Protocatechuic	41.2 ± 2.00[b]	44.4 ± 0.93[a]	0.61 ± 0.02[e]	nd	20.5 ± 0.89[c]	9.55 ± 0.95[d]
Vanillin	5.36 ± 0.36[ab]	5.70 ± 0.10[a]	6.13 ± 0.49[a]	2.62 ± 0.29[bc]	1.82 ± 0.11[cd]	1.30 ± 0.19[d]
Ferulic	< LQ	2.95 ± 0.23[e]	4.23 ± 0.10[d]	54.9 ± 3.36[a]	48.7 ± 0.29[b]	13.6 ± 0.32[c]
Gallic	2.29 ± 0.07[d]	< LQ	3.21 ± 0.22[c]	23.2 ± 0.48[a]	6.06 ± 0.54[b]	< LQ
p-Coumaric	2.28 ± 0.14[b]	< LQ	0.34 ± 0.03[d]	1.84 ± 0.21[c]	3.14 ± 0.27[a]	< LQ
Syringic	nd	1.97 ± 0.22[bc]	1.10 ± 0.09[cd]	0.92 ± 0.15[de]	7.55 ± 0.82[ab]	42.3 ± 2.94[a]
Σ acids	453 ± 21.7[a]	95.2 ± 1.08[bc]	45.2 ± 1.00[c]	117 ± 3.25[ab]	95.0 ± 0.97[bc]	92.4 ± 4.12[c]
Quercetin	nd	nd	4.25 ± 0.21[c]	18.8 ± 1.91[a]	nd	8.28 ± 0.34[b]
FPC (mg GAE g^{-1}) *	16.5	11.8	24.4	14.6	15.9	9.57 ± 0.16

Σ acids - Sum of mean concentration of all phenolic acids. FPC - Free Phenolic Compounds. < LQ - Below limit of quantification. nd – non detected. Values with different letters are significantly different among the species shown in rows. * Average FPC data published in Souza et al., (*in press*), except *Vigna luteola* content that was estimated in the present study.

Table 3. DPPH radical scavenging activity (%I_{DPPH}) for *A. graveolens*, *M. parvifolia*, *P. vaginatum*, *S. neei*, *S. terebinthifolius* and *V. luteola.* For three different extract concentration every ten minutes for one hour. Analysis of variance (ANOVA) for repeated measure results, consider significance $p < 0.025$, are present too.

		A. graveolens			*M. parvifolia*			*P. vaginatum*		
		A	B	C	A^b	B^a	C^a	A	B	C
Time (minutes)	0	83.1 ± 0.9	80.4 ± 5.1	82.7 ± 1.5	88.6 ± 0.6	85.6 ± 1.9	91.9 ± 0.1	79.5 ± 8.4	84.9 ± 1.8	87.1 ± 2.3
	10	85.0 ± 0.6	82.4 ± 4.9	83.2 ± 1.3	89.8 ± 0.6	85.7 ± 2.2	92.1 ± 0.1	79.8 ± 7.4	85.2 ± 2.2	87.9 ± 2.2
	20	84.9 ± 1.0	82.9 ± 4.4	83.3 ± 0.1	90.0 ± 0.4	84.8 ± 2.4	91.4 ± 0.6	79.6 ± 7.2	84.8 ± 2.2	87.0 ± 2.0
	30	84.8 ± 0.6	82.4 ± 3.9	83.2 ± 0.9	88.6 ± 0.7	84.2 ± 2.2	91.6 ± 0.6	78.8 ± 6.8	84.8 ± 1.1	86.6 ± 1.8
	40	84.7 ± 0.6	82.5 ± 3.5	82.6 ± 1.2	88.8 ± 0.7	83.6 ±2.3	91.5 ± 0.6	78.1 ± 5.8	84,1 ± 0.6	85.8 ± 1.7
	50	84.8 ± 0.4	82.4 ± 3.6	82.3 ± 0.9	88.6 ± 0.7	83.9 ± 2.5	91.7 ± 0.7	77.4 ± 5.1	84.2 ± 0.6	85.5 ± 1.7
	60	84.7 ± 0.3	82.5 ± 3.4	81.6 ± 1.1	88.4 ± 1.0	83.2 ± 2.5	91.5 ± 0.7	79.9 ± 5.7	84.2 ± 0.8	85.3 ± 2.1
		ANOVA for repeated measures								
		SS	F	p	SS	F	p	SS	F	p
C		0.30	0.85	0.493	3.35	36.6	***< 0.025***	0.22	3.06	0.223
T		0.07	6.37	0.082	0.09	20.3	0.036	0.02	9.75	0.078
C x T		0.04	3.39	0.181	0.57	10.6	0.026	0.03	0.49	0.618
Residue		1.19			0.19			0.16		

		S. neei			*S. terebinthifolius*			*V. luteola*		
		A^a	B^a	C^b	A	B	C	A	B	C
Time (minutes)	0	66.6 ± 1.9	64.9 ± 0.7	41.2 ± 2.9	55.4 ± 0.7	94.7 ± 1.0	71.4 ± 17.5	79.5 ± 5.2	67.6 ± 4.2	53.3 ± 4.9
	10	78.5 ± 2.8	79.4 ± 1.2	48.6 ± 1.0	56.7 ± 4.1	95.5 ± 1.2	72.6 ± 18.7	86.7 ± 3.5	79.3 ± 4.0	68.1 ± 1.7
	20	82.4 ± 0.9	85.3 ± 0.6	54.4 ± 2.8	54.9 ± 3.2	95.9 ± 2.1	71.5 ± 19.4	88.1 ± 2.9	84.8 ± 3.4	78.9 ± 0.9
	30	83.1 ± 0.6	91.8 ± 1.1	62.1 ± 2.2	51.8 ±2.7	96.8 ± 1.4	71.7 ± 19.9	88.1 ± 2.6	88.1 ± 3.3	83.5 ± 1.5
	40	83.9 ± 0.5	95.7 ± 0.9	64.9 ± 0.1	49.7 ± 0.4	98.0 ± 2.8	71.1 ± 19.7	88.0 ± 2.3	89.3 ± 3.0	85.8 ± 1.7
	50	84.3 ± 0.5	98.5 ± 0.8	69.4 ± 0.0	48.4 ± 1.4	95.7 ± 4.1	68.6 ± 18.6	87.9 ± 2.4	89.8 ± 2.8	87.8 ± 0.9
	60	84.8 ± 0.5	99.3 ± 0.8	71.5 ± 0.0	47.7 ± 0.1	94.6 ± 4.1	67.5 ± 19.0	87.8 ± 2.1	89.3 ± 2.6	88.6 ± 0.8
		ANOVA for repeated measures								
		SS	F	p	SS	F	p	SS	F	p
C		44.1	397	***< 0.025***	0.08	11.8	0.076	3.46	4.21	0.104
T		25.1	621	***< 0.025***	0.001	9.32	0.085	14.8	179	***< 0.025***
C x T		4.08	45.0	***< 0.025***	0.001	10.6	0.040	2.81	31.1	***< 0.025***
Residue		0.48			0.01			1.99		

A, B and C are the concentration of extracts of the 19, 14 and 10 mg dw of plant per mL^{-1}, respectively. For the *S. neei*, this concentration is 10, 7 and 5 mg dw mL^{-1}, respectively. C- Concentration; T- Time; SS- Sums squares; F- F calculated. Values with different letters are significantly different among the concentrations shown in rows.

Table 4. Inhibition of peroxidase enzyme (PO) activity (%I_{PO}) for *Apium graveolens*, *Myrsine parvifolia*, *Paspalum vaginatum*, *Salicornia neei*, *Schinus terebinthifolius* and *Vigna luteola.* For three different extract concentration every ten minutes for one hour. Analysis of variance (ANOVA) for repeated measure results are present too.

	Apium graveolens				*Myrsine parvifolia*				*Paspalum vaginatum*			
	A	B	C	D	A[a]	B[ab]	C[b]	D[ab]	A	B	C	D
Time (minutes) 10	55.9 ± 7.6	-	64.7 ± 3.2	33.5 ± 34.0	48.0 ± 5.0	72.0 ± 28.0	-72.3 ± 13.3	45.8 ± 38.2	52.2 ± 8.1	-	71.7 ± 12.4	9.89 ± 62.9
20	38.9 ± 4.4	-	62.1 ± 0.3	28.7 ± 26.0	46.2 ± 5.8	74.1 ± 29.4	70.5 ± 10.0	36.6 ± 25.0	48.5 ± 7.6	-	55.5 ± 1.8	-7.93 ± 55.8
30	43.5 ± 1.6	-	67.0 ± 8.1	22.8 ± 33.1	46.4 ± 5.8	74.2 ± 24.2	-68.9 ± 10.2	31.5 ± 22.7	51.2 ± 10.0	-	43.3 ± 9.6	-18.1 ± 57.8
40	53.7 ±3.7	-	63.9 ± 5.3	22.8 ± 36.0	47.5 ± 5.3	78.2 ± 21.6	-63.9 ± 11.6	20.3 ± 43.6	84.3 ± 4.5	-	41.8 ± 5.4	-20.9 ± 59.5
50	64.8 ± 8.6	-	61.5 ± 0.2	22.2 ± 30.4	49.4 ± 5.0	82.9 ± 19.3	-58.4 ± 12.3	21.7 ± 45.1	88.1 ± 15.3	-	41.0 ± 5.2	-21.0 ± 60.7
60	69.5 ± 2.1	-	63.6 ± 1.2	24.4 ± 29.1	51.7 ± 5.4	87.6 ± 17.8	-54.3 ± 12.9	27.7 ± 41.1	66.0 ± 2.07	-	42.5 ± 6.5	-19.9 ± 61.7

ANOVA for repeated measures										
	SS	F	p		SS	F	p		SS	F	p
C	0.19	3.79	0.12	C	1.92	18.2	***< 0.025***	C	0.56	5.38	0.15
T	0.01	6.49	0.10	T	0.00	0.88	0.45	T	0.02	3.77	0.19
C x T	0.03	7.14	0.06	C x T	0.03	2.59	0.24	C x T	0.07	7.28	0.11
Residue	0.11			Residue	0.24			Residue	0.24		

A, B, C and D are the concentration of extracts of the 16, 12, 8 and 4 mg mL^{-1}, respectively. For the *S. neei*, this concentrations is 8, 6, 4 and 2 mg mL^{-1}, respectively. C- Concentration; T- Time; SS- Sums squares; F- F calculated. Values with different letters are significantly different among the concentrations shown in rows.

Table 4 (continuation). Inhibition of peroxidase enzyme (PO) activity (%I_{PO}) for *Apium graveolens*, *Myrsine parvifolia*, *Paspalum vaginatum*, *Salicornia neei*, *Schinus terebinthifolius* and *Vigna luteola.* For three different extract concentration every ten minutes for one hour. Analysis of variance (ANOVA) for repeated measure results are present too.

	Salicornia neei				*Schinus terebinthifolius*				*Vigna luteola*			
	A[a]	B[a]	C[a]	D[a]	A	B	C	D	A[ac]	B[bc]	C[c]	D[bc]
Time (minutes) 10	26.6 ± 18.0	39.3 ± 30.1	-88.9 ± 11.1	-73.2 ± 26.8	98.4 ± 6.3	93.1 ± 13.2	95.8 ± 9.1	97.6 ± 4.9	-19.6 ± 4.4	-86.1 ± 13.9	-93.8 ± 10.7	-49.3 ± 8.62
20	-6.26 ± 5.9	-48.0 ± 43.0	-53.8 ± 19.0	-70.1 ± 8.3	94.2 ± 4.8	95.2 ± 10.2	97.5 ± 5.5	98.6 ± 2.6	-15.8 ± 0.2	-30.0 ± 77.9	-95.4 ± 7.9	-36.5 ± 7.86
30	-0.96 ± 0.5	-47.7 ± 37.2	-85.4 ± 14.6	-66.1 ± 13.7	91.2 ± 4.7	95.0 ± 9.6	97.8 ± 3.9	98.4 ± 2.5	-8.25 ± 7.6	-29.6 ± 5.9	-88.8 ± 19.4	-38.2 ± 4.22
40	47.3 ± 0.7	-42.7 ± 39.1	-83.5 ± 16.5	-61.5 ± 8.8	89.0 ± 4.6	92.2 ± 10.8	97.9 ± 3.1	98.0 ± 2.8	40.3 ± 20.6	-27.8 ± 5.4	-89.9 ± 17.6	-37.1 ± 3.95
50	17.9 ± 2.3	43.6 ± 16.4	-48.1 ± 15.1	-55.6 ± 10.1	87.6 ± 4.9	90.3 ± 12.1	97.7 ± 2.8	97.8 ± 2.8	22.7 ± 3.8	-24.0 ± 5.0	-90.2 ± 17.0	-35.4 ± 2.37
60	7.62 ± 2.6	84.0 ± 2.3	-45.5 ± 14.6	-53.3 ± 10.5	86.9 ± 5.5	88.4 ± 13.3	97.6 ± 2.6	97.9 ± 2.9	14.8 ± 4.4	-19.6 ± 4.4	-90.7 ± 16.2	-33.4 ± 1.76

ANOVA for repeated measures											
	SS	F	p		SS	F	p		SS	F	p
C	140	28.1	***< 0.025***	C	35.2	1.29	0.37	C	0.33	45.9	***< 0.025***
T	28.4	12.1	0.04	T	4.36	3.06	0.22	T	0.04	76.6	***< 0.025***
C x T	44.2	8.78	0.04	C x T	9.64	6.35	0.10	C x T	0.05	24.9	***< 0.025***
Residue	24.7			Residue	60.3			Residue	0.00		

A, B, C and D are the concentration of extracts of the 16, 12, 8 and 4 mg mL^{-1}, respectively. For the S. neei, this concentrations is 8, 6, 4 and 2 mg mL^{-1}, respectively. C- Concentration; T- Time; SS- Sums squares; F- F calculated. Values with different letters are significantly different among the concentrations shown in rows.

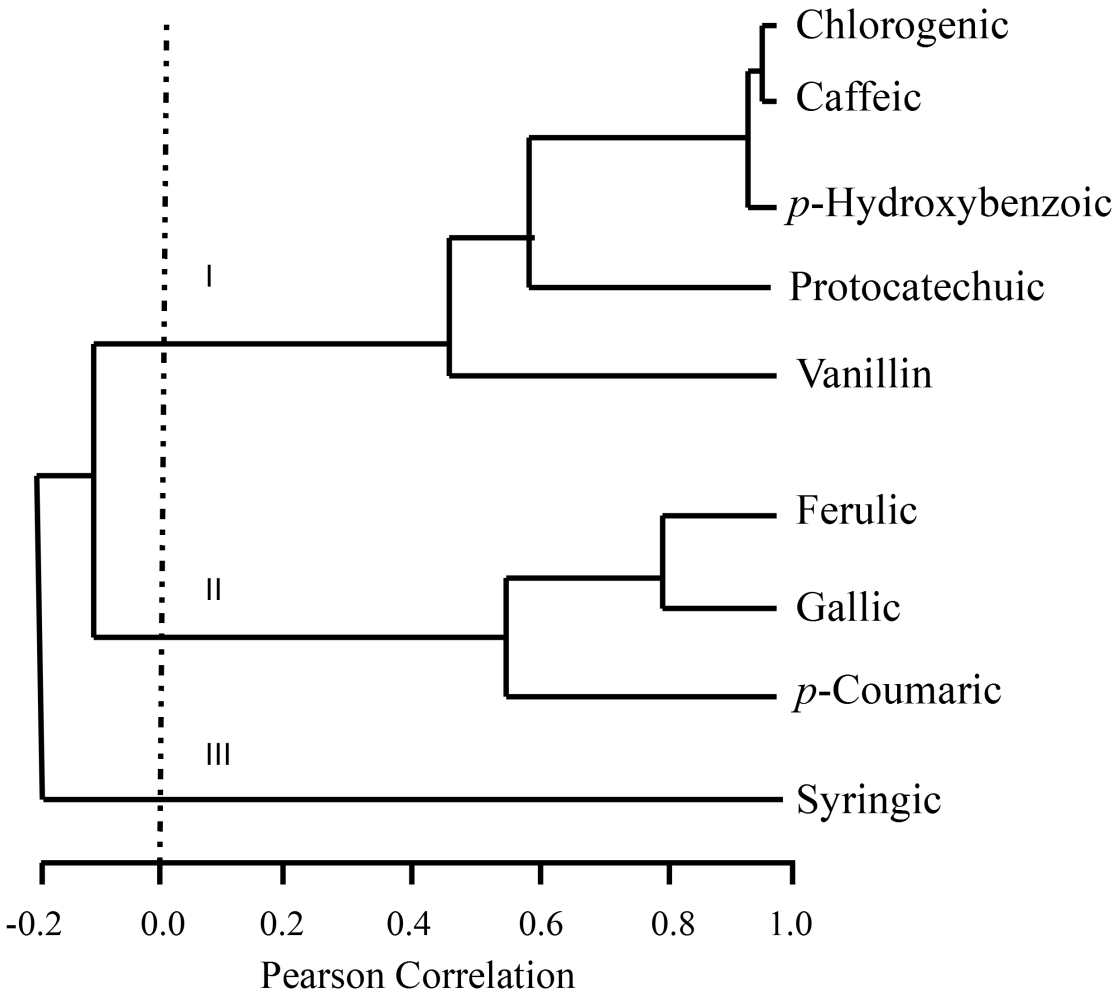


Figure 1. Cluster analysis of phenolic acids contents in leaf/shoot tissues of Brazilian halophytes. Dashed line separates three groups of phenolic acids by negative weighted average intergroup correlations.

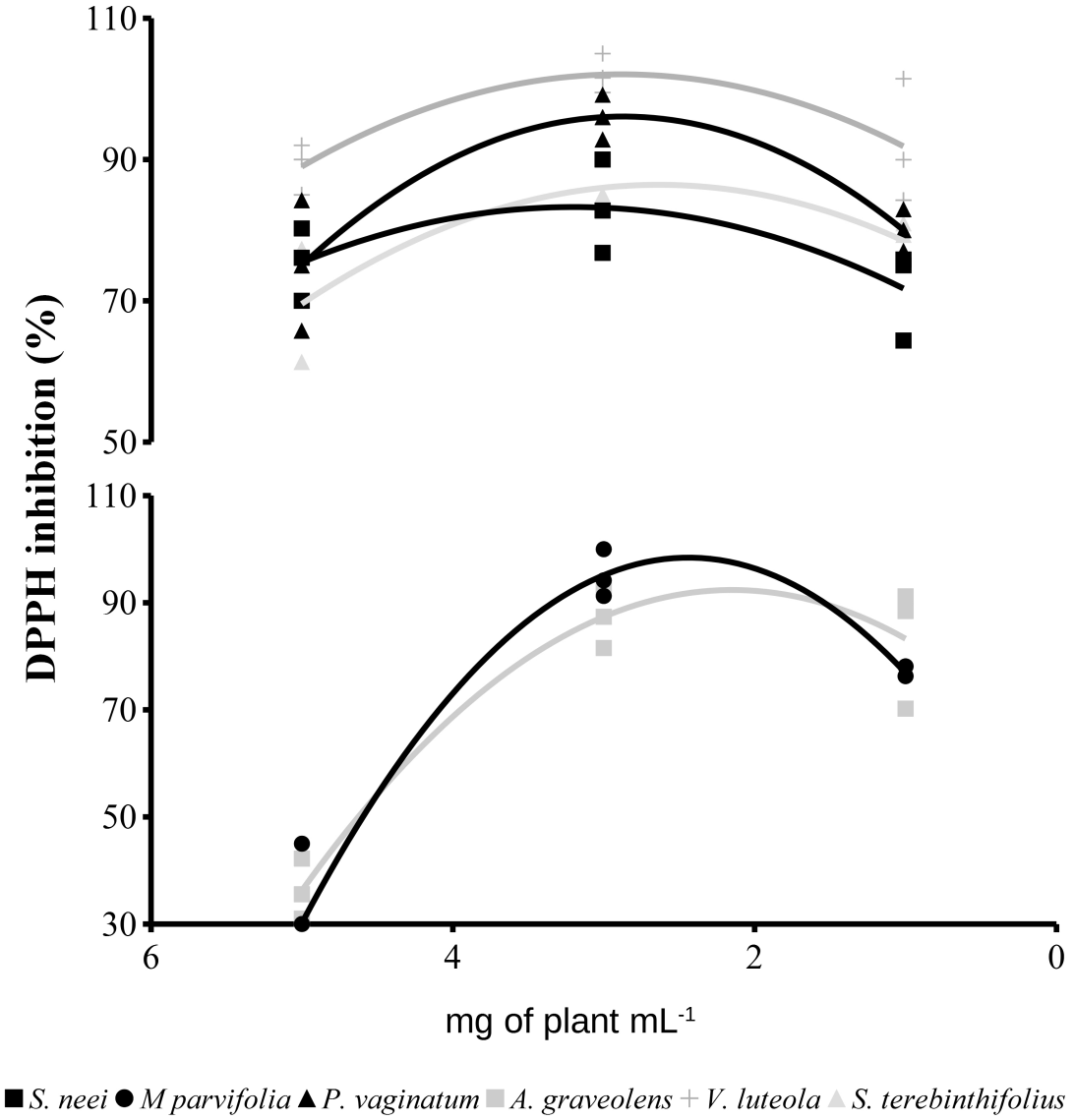


Figure 2. Percentual inhibition of DPPH radicals by ethanolic extracts with different dilutions of the Brazilian halophytes *A. graveolens*, *M. parvifolia*, *P. vaginatum*, *S. neei*, *S. terebinthifolius* and *V. luteola*. Ploted curves are predicted values obtained by quadratic model for each halophyte species. Points are triplicate values of diluted extracts determination.

Capítulo 3

Growth, phenolic compounds, photosynthetic pigment composition and antioxidant responses of two new genotypes of the sea asparagus *Salicornia neei* to salt stress in greenhouse and field conditions.

Manuel Macedo de Souza[1], Carlos Rafael Borges Mendes[2], Kennia Brum Doncato[1], Eliana Badiale-Furlong[3], César Serra Bonifácio Costa[1*].

[1]Laboratório de Biotecnologia de Halófitas (BTH), Programa de Pós-Graduação em Aquicultura, Instituto de Oceanografia (IO), Universidade Federal do Rio Grande – FURG, Av. Itália km 8, 96203-900 Rio Grande, RS, Brazil;

[2]Laboratório de Fitoplâncton e Microorganismos Marinhos, Instituto de Oceanografia, Universidade Federal do Rio Grande (FURG), Av. Itália, km 8, Rio Grande, RS 96203-900, Brazil

[3]Laboratório de Micotoxinas e Ciência de Alimentos, Programa de Pós-Graduação em Engenharia e Ciência de Alimentos, Escola de Química e Alimentos (EQA), FURG.

* Corresponding author.

E-mail adresses: mcsouza@furg.br (M.M. Souza), dqmebf@furg.br (E. Badiale-Furlong), crbmendes@gmail.com (C.R.B. Mendes), kenniadoncato@hotmail.com (K.B. Doncato), docosta@furg.br (C.S.B. Costa)*.

RESUMO

Salicornia neei Lag. é uma espécie de aspargo marinho nativa da América do Sul com inúmeras propriedades bioativas e com grande potencial para produção de biomassa sob irrigação com efluente salino aquícola. O objetivo deste estudo foi comparar os efeitos da exposição ao estresse salino no crescimento, concentrações de pigmentos fotossintéticos, composição de compostos fenólicos e atividade antioxidante dos caules de dois genótipos (BTH1 e BTH2) de *S. neei*. Durante 50 dias plantas foram cultivadas em estufa sob diferentes salinidades (0, 34.2, 85.5, 171, 513 e 769 mM NaCl) ajustadas em solução nutritiva integral de Hoagland. Em um segundo experimento, plantas também foram cultivadas por 154 dias em parcela de campo irrigada com efluente salino de carcinicultura (213 mM NaCl). O genótipo BTH1 apresentou melhor desempenho no experimento em estufa, mas foi mais afetado pelo aumento das salinidades do que o genótipo BTH2. Ambos os genótipos de *S. neei* apresentam alta tolerância ao estresse salino, com mecanismos distintos contra o estresse foto oxidativo. O genótipo BTH1 utilizou seu alto teor de carotenoides para dissipar o poder oxidativo. Exposto a salinidades crescentes, o genótipo BTH2 aumentou o teor relativo da clorofila b para reduzir a taxa de foto-oxidação e elevou seu conteúdo do flavonoide quercetina para aumentar a ação antioxidante. No experimento de campo, BTH2 apresentou melhor desenvolvimento, com maior crescimento, produção de metabólitos e capacidade antioxidante do que o genótipo BTH1 e também culturas tradicionais irrigadas com água doce. Nossos resultados mostraram que o genótipo BTH2 de *S. neei* poderia ser uma boa alternativa para a produção de alimentos ricos em compostos bioativos utilizando águas/efluentes salinos.

Palavras-chave: Halófitas; Estresse salino; Alimento funcional; Efluente de carcinicultura; Agricultura salina.

ABSTRACT

Salicornia neei Lag. is a native South American species of sea asparagus with great potential for biomass production and high bioactive properties under irrigation with aquaculture saline effluent. This study aimed to compare the effects of salt stress exposition on growth, concentrations of photosynthetic pigments, phenolic compounds composition and antioxidant activity of the shoot biomass of two genotypes (BTH1 and BTH2) of *Salicornia neei*. Plants were cultivated during 50 days in a greenhouse exposed to different salinities (0, 34.2, 85.5, 171, 513, and 769 mM NaCl) adjusted in full strength Hoagland solutions, as well as for 154 days in a field plot irrigated with saline shrimp farm effluent. BTH1 genotype showed better performance in the greenhouse experiment but it was more affected by increasing salinities than BTH2 genotype. Both *S. neei* genotypes have high salt tolerance, with distinct mechanisms against photoxidative stress. Under increasing salt stress, BTH2 genotype increased the relative content of chlorophyll *b* to lower photo-oxidation rate and raised shoot content of the flavonoid quercetin for enhancement of antioxidant action. BTH1 genotype used its high carotenoids content to dissipate the oxidative power. In the field experiment BTH2 showed a better development, with higher growth, more production of metabolites and, consequently, a greater antioxidant capacity than both BTH1 genotype and traditional crops irrigated with fresh water. Our results showed that *S. neei* BTH2 genotype could be a good alternative for production of a food rich in bioactive compounds using saline waters/effluents.

Keywords: Halophyte; Salinity stress; Functional food; Shrimp farm effluent; Salinity agriculture.

1. Introduction

Plants of the genus *Salicornia* and *Sarcocornia* (Salicornioideae, Amaranthaceae) are small succulent shrubs with leafless stems and branches commonly named sea asparagus, glasswort or samphire (Rozema & Schat, 2013; Patel, 2016). The most recently worldwide phylogenetic treatment of Salicornioideae based on molecular markers Piiranen et al., (2017) proposed merging of the genus *Sarcocornia* in three subgenera under *Salicornia*. These plants are halophytes found worldwide in salt marshes and salt deserts, which are consumed as a green salad, in gourmet food, as conserves, and beverages (*nuruk*, wine and vinegar) (Patel, 2016; Ventura, Eshel, Pasternak, & Sagi, 2015). Cultivation of sea asparagus has been performed in different parts of the world (e.g. U.S.A., México, Israel, Saudi Arabia, Eritrea and Brazil) with aquaculture saline effluent, and brackish and/or seawater as experimental and commercial crops (Doncato & Costa, 2017; Patel, 2016; Ventura et al., 2011). In general sea asparagus showed high yields in a wide range of salinities but each species present its own singular grow responses under increasing saline stress (Bresdin, Glenn, & Brown, 2016).

Besides the high content of crude protein, fatty acids (e.g. oleic, linoleic and palmitic acids) and minerals (Barreira et al., 2017; Bertin et al., 2014; Costa et al., 2014; Doncato & Costa, 2017), sea asparagus shoots are also rich in organic metabolites with bioactive properties, such as phenolic acids (e.g. caffeic, ferulic, and syringic), flavonoids (e.g. quercetin and kaempferol) (Bertin et al., 2014; Costa et al., 2018; Oh, Kim, Lee, Woo, & Choi, 2007), vitamin C (Costa et al., 2018) and carotenoids (Barreira et al., 2017; Costa et al., 2006). Consequently, the consumption of these plants can produce numerous health benefits to humans, such as protection against oxidative stress, incite immune responses, prevention of weight gain and accumulation of lipids (Patel, 2016; Rahman et al., 2018). Production of organic functional compounds by sea asparagus, as for other halophytes plants, mainly results from their physiological adaptations to survive under salt stress and its associated effects (e.g., ionic, osmotic and oxidative stress) (Flowers & Colmer, 2015; Hasegawa, Bressan, Zhu, & Bohnert, 2000). Thus, in order to commercially produce sea asparagus with desirable agricultural

traits, it is important to understand how salt cultivation conditions affect productivity and chemical composition of these halophytes.

The saline environment creates several ionic, osmotic and oxidative stresses, and in general, halophytes present tolerance mechanisms based on ions bombs (for ionic stress), salt compartmentalization in vacuole and organic osmolite production in the cytoplasm (for osmotic stress), adjustments of the photosynthetic apparatus and production of antioxidant molecules (for oxidative stress) (Duarte et al., 2013; Flowers & Colmer, 2015; Redondo-Gómez et al., 2006). Oxidative stress results from stomata closure under salt exposure and inability of the plant reduce electrons generated by photosynthesis in light phase, due to the lacking of CO_2 molecules, what can lead-induced the reducing power (electrons) acts on other molecules generating reactive oxygen species (ROS). The photosynthetic pigments play an important role in preventing and combating oxidative stress in the photosystem (photooxidative stress). To avoid damage, chlorophyll b content may be favoured by having a protective function of the photosystem, due to the lower photo-oxidation rate than chlorophyll a (Dubey, 2005). Furthermore, carotenoids can also be activated in the extinction of electrons by transfer of excitation and harmless thermal dissipation (xanthophyll cycle). In addition, carotenoids acting as antioxidant molecules against ROS generated if the intervention mechanisms are not efficient (Duarte et al. (2013); Latowski, Kuczyńska, & Strzałka, 2011; Taiz & Zeiger, 2002). Besides that, several halophytes showed their content of phenolic compounds directly proportional to the intensity of salt stress (Bertin et al. 2014; Costa et al. 2006; Fan et al. 2013; Ventura et al. 2011) and the presence of these compounds frequently linked with scavenger actions against ROS (Costa et al., 2018; Duarte et al., 2013).

Since 2010, the Biotechnology Halophyte Laboratory (BTH Lab) of the Federal University of Rio Grande (FURG; Brazil) has being carried out a breeding program that generated two distinctive pure genotypes of southern Brazil plants of *Salicornia neei* Lag., denominated BTH1 (prostate shoot growth and red phenotype at maturity) and BTH2 (decumbent shoot growth and green phenotype at maturity) (Costa et al., 2018; Doncato & Costa, 2017; Freitas & Costa, 2014). *Salicornia neei* is a native South American species (previously named *Salicornia gaudichaudiana* Moq. and *Sarcocornia ambigua* (Michx.) M.A.Alonso & M.B.Crespo) and, besides their distinct shoot

morphology, the two new genotypes show differences at molecular level (Costa, Kadereit, & Freitas, *in press*), in their biomass production, mineral and phenolic composition under irrigation with aquaculture saline effluent (Costa et al., 2018; Doncato & Costa, 2017).

This study aimed to evaluate the effects of salt stress exposition on growth, concentrations of photosynthetic pigments, phenolic compounds composition and antioxidant activity of the shoot biomass of two genotypes (BTH1 and BTH2) of *Salicornia neei*. These aims were accomplished through greenhouse and field trials, and growth performance and biochemical composition of *S. neei* were also compared with traditional food plants.

2. Material and methods

2.1. Plant material and growth conditions

The seeds of *S. neei* were obtained from germplasm of BTH Lab (Institute of Oceanography, FURG, Rio Grande, Brazil). Seeds of f3 progenies of BTH1 and BTH2 genotypes of *S. neei* were germinated at thermoperiod of 12 h (30 ºC): 12 h (20 ºC) (Freitas & Costa, 2014). Seedlings were transferred to Styrofoam trays filled with a mixture (1: 1) of an organic compound (Humosolo Vida®) and fine sand beach and, 6 weeks later, to 50 cm^3 plugs with the same substrate. Seedlings were maintained for up to 38 weeks irrigated with tap water in non-heated greenhouse located in Institute of Oceanography of FURG (32º04’43’’S; 52º10’03’’W) before the experiments.

2.1.1. Greenhouse experiment

Thirty-four weeks-old plants of the two *S. neei* genotypes were planted in a solution culture system. Individual plants were transferred into 50 cm^3 polyethylene plugs filled with washed fine sand beach. The plugs were placed into holes of a rack suspended over a 31 cm x 12 cm x 18 cm polyethylene trays, which were filled with full-strength Hoagland solutions. For each *S. neei* genotype, a total of six trays were used each holding 24 plugs (replicate plants). Five trays were subjected to salinity levels, and one tray was maintained as control (with no salt addition). Salinity levels

were obtained by adding commercial marine salt Cisne® to full strength Hoagland solutions and salinity levels, consisting of 0 (control), 34.2, 85.5, 171, 513, and 769 mM NaCl. Salt treated plants were initially keep for two weeks in 34.2 mM NaCl (acclimation period) before the salt concentration of each treatment level be raised until it full strength. Solutions were changed weekly and plug sands keep close to the maximum holding capacity. The experimental had a randomized complete block design and it was carried out in non-heated greenhouse. The duration of the treatment was 50 days, during the austral summer (December 2014 – January 2015). The average conditions inside greenhouse were: temperature 31.7 ± 3.2/20.1 ± 2.8 ºC maximum/minimum; irradiation 6.83 ± 2.22 MJ m^{-2} day^{-1}; and air humidity 72.2 ± 12.9 %. Temperature was measured with a mercury thermometers, and a meteorological station ofs INMET (Institute National of Meteorology, Brazil), located in FURG campus, was used to obtain the values of irradiation and humidity data. Pots were randomly re-located every two weeks.

For each plant, shoot height (cm) and branch number (only primary-first order branches) were quantified at the beginning (after acclimation period) and at the end of the experiment. Branch formation was calculated by the difference between initial and final values of branch number. Individual fresh (FW) and dry (DW) weights (g) were obtained from an extra lot of 10 plants keep in control solution. Dry weight was estimated by freeze-drying (lyophilized; -50 ºC for 48 hours) for better maintaining biomass quality. Dry weight estimation by freeze-drying and oven-drying (60 ºC) showed no statistical difference (data not published). The succulence was defined as the water content in shoots and estimated by the percent difference between fresh and dry weight {[(FW – DW)*100] / FW}. Relative shoot dry weight (RDW) was estimated to determining salinity effect in dry biomass production. For each salinity level, RDW was expressed as percentage of the average biomass of control treatment (0 mM NaCl) biomass.

2.1.2. Field experiment

Thirty-eight weeks old plants of *S. neei* BTH1 and BTH2 genotypes were planted in two separated 1.6 m x 3.5 m subplots established inside a larger plot (6.5 x 3.5 m) localized in the Marine Aquaculture Station, FURG (32º 12' 19''S; 52º 10'45''W). Plot design and soil characteristics are described in Doncato & Costa (2017). Between November 2014 and April 2015 plants were watered every other day by filling up drainage ditches with 375 L of saline effluent from shrimp farm (*Litopenaeus vannamei*) with the Biofloc Technology System, stocked with 87 shrimp m^{-2}. Average environmental conditions (effluent, soil and meteorological) during a field experiment are shown in Table 1.

After 154 days (22 weeks) of field cultivation, plants were cut just above ground level, washed to remove soil and had their shoot height measured with a ruler (mm) and branch number quantified. Shoot were weighed on a precision scale to determine the fresh weight (FW). Five plants of each *S. neei* variety were randomly chosen, rinsed with distilled water and frozen at -20 ºC. These samples were lyophilized (-48 ºC during 48 hours), dry weight (DW) estimated in a precision scale, and biomass ground in knife mills. Subsequently, succulence was estimate according equation previously presented. Initial fresh and dry weight were estimated by the linear relationship between height and fresh (and dry) biomass obtained by an extra lot of plants collected at the planting date.

2.2. Plant metabolites and free-radical scavenging capacity of shoot extracts

Plant of both *S. neei* genotypes cultivated at different salinity levels inside the greenhouse and at the field plot irrigated with saline effluent had their shoot metabolites quantified. The chemical analyses were performed in triplicate with the homogeneous fresh sample freeze-drying shoots.

2.2.1. Photosynthetic pigments

The photosynthetic pigments were determined only for shoot samples from the greenhouse experiment following the method recommended by Mendes, Cartaxana, & Brotas (2007). The samples were sonicated for 5 mi in an ice-water bath, placed at

-20ºC for 1 h, and then centrifuged at 1100 g for 5 min at 3ºC. The supernatants were filtered through fluoropore PTFE membrane filters (0.2 µm pore size; Merck Millipore Ltd, Billerica, MA, USA), diluted (1 mL of sample was mixed with 0.40 mL of ultrapure water) in 2 mL amber glass sample vials, and immediately placed in the HPLC cooling rack (4ºC). Pigment extracts were analysed in a Shimadzu HPLC (Shimadzu Corporation, Kyoto, Japan) using monomeric C8 column (SunFire; 15 cm, 4.6 mm, 3.5 µm) with a pyridine-containing mobile phase. The HPLC system was calibrated with pigments standards from DHI (Institute for Water and Environment, Denmark). The determinations were done as triplicate, and results were expressed in µg g^{-1} dry weight.

In order to evaluate the light harvesting and photo-protection mechanisms the ratios of chlorophyll *a*:*b* and the total chlorophyll to total carotenoids, as well as the De-epoxidation state of xantophylls (DES), were calculated (Costa et al., 2006; Duarte, Santos, Marques, & Caçador, 2013).

2.2.2. Phenolic compounds and antioxidant activity

The extractions of phenolic compounds were carried in triplicate by ultrasound extraction assisted and free phenolic compounds (FPC) were measured by the Folin-Ciocalteau method, using a spectrophotometer at 750 nm (Biospectro, SP-22, Brazil) (Souza, da Silva, Costa, & Badiale-Furlong, *in press*). The concentration of FPCs was estimated using a standard curve of gallic acid (2.2 to 22 µg mL^{-1}) and free phenolic content was expressed as milligram of gallic acid equivalents (GAE) per gram of dry weight sample (mg GAE g^{-1} dw). Free flavonoid compounds (FFC) were measured according to Gajula et al. (2009). 0.11 mL of 80% ethanol was added in 0.16 mL of extract, followed by the addition of 0.08 mL of $AlCl_3$ solution (5% w/v). After thirty minutes was realized the spectrophotometric determination in 405 nm (Reader TP thermoplate nm). FFCs was estimated using a standard curve of quercetin (0.62 to 10 µg mL^{-1}) and free flavonoid content was expressed as milligram of quercetin equivalents (QE) per gram of dry weight sample (mg QE g^{-1} dw).

The identification and quantification of phenolic acids and quercetin in the extracts were carried out by HPLC-UV-Vis (Shimadzu LC-10), equipped with a

Discovery Bio Wide Pore C18 column (5 µm, 25 cm x 4.6 mm) (adapted of the Scaglioni, de Souza, Schmidt, & Badiale-Furlong, 2014). The extract was mixed with mobile phase (1:1) and centrifuged at 1400 *g* (Eppendorf Centrifuge 5804 R, Hamburg, Germany) prior to injection. The HPLC-UV-Vis operated at a flow rate of 0.7 mL min^{-1}, at 35 ºC, using a gradient mobile phase consisting of methanol and acidified ultrapure water (glacial acetic acid 1%) (0 min = 30%; 5 min = 40%; 10 min = 50%; 15 min = 60%, and 20 min = 30% methanol; v/v) for 30 min. The wavelength utilized was 280 nm, changing to 320 nm between 14 and 25 min. The contents of phenolic acids and quercetin were expressed as microgram per gram of dry weight sample (µg g^{-1} dw).

The free-radical scavenging capacity of each extract was investigated against DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical, and estimated according to the method of Nicklisch & Waite (2014) adapted. 10 µL of ethanolic DPPH solution (0.80 mg mL^{-1}) were added to 190 µL of extract iin different concentrations (10, 7 and 5 mg plant dw mL^{-1}). Absorbance was measured at 490 nm (TP Reader NM Thermoplate) at intervals of 10 min until 1 h. Measurements started immediately after mixing the solutions.

The analyses were performed in triplicate and DPPH inhibition percentage ($\%I_{DPPH}$) was estimated. For each salinity level of the greenhouse experiment and also for field cultivated plants was determined the plant extract concentration necessary to produce 50% I_{DPPH} (IC_{50}), estimated by linear regressions (extract concentrations vs. $\%I_{DPPH}$).

2.3. Statistical analysis

All results were expressed as mean and standard deviation. Two-way ANOVA was used to evaluate the effect of salinity and genotype identity (and their interaction) on growth, concentrations of photosynthetic pigments, phenolic compounds and antioxidant activity of *S. neei* shoot biomass. The ANOVAs were followed with a comparison by Tukey's HSD test. Normality of residues and homoscedasticity were tested by Shapiro-Wilks and Bartlett test, respectively. For the best adjustment to the assumptions of ANOVA the data (of greenhouse experiment) were transformed by $x^{0.5}$ (branch number, syringic acid content and DES), $x^{7.4}$ (succulence), $x^{0.45}$ (violaxanthin content), x^{-1} (zeaxanthin content) and ln(x) (lutein, neoxanthin and sum of carotenoids).

For each genotype, Pearson's correlations were performed to search for possible relationships among metabolites´ contents (pigments and phenolic compounds) and antioxidant activity (50% I_{DPPH} – IC50) in each genotype. Field data of growth, metabolites´ concentrations and antioxidant activity of the two *S. neei* genotypes were analysed by Student's t-test.

For all statistical analysis, non-detected metabolic parameters values were replaced by the limit of detection of each parameter, and the significance value considered was $p < 0.05$. All statistical analyses were performed in R software.

3. Results

3.1. Responses of S. neei genotypes to different salinity levels (greenhouse experiment)

3.1.1. Growth responses

The average initial shoot height, branch number, fresh and dry weights of *S. neei* genotypes were 11.4 ± 3.6 cm, 2.2 ± 3.3, 0.30 ± 0.20 g and 0.07 ± 0.02 g, respectively. Except for shoot branch number (3.5 ± 3.7 and 0.9 ± 2.2 branches per shoot for BTH1 and BTH2, respectively), the initial values of plant attributes did not show statistical differences among salinity levels and between plant genotypes. After 50 days of cultivation in different salinities, no plant death was observed for *S. neei* genotypes. Shoot height did not significantly differ between *S. neei* genotypes and among salinity levels. Final shoot branch number of BTH1 genotype was twice larger than BTH2 plants but neither genotype had its branch number significantly affected by salinity (Table 2).

Salicornia neei genotypes showed similar final shoot FW and DW, but only shoot FW was significantly affected by salinity. Genotypes showed different responses to salinity stress (significant interaction genotype X salinity), being BTH1 plants grown at 86 mM NaCl heavier than those subjected to higher salinity (769 mM NaCl), whereas BTH2 FW was not affected by salinity (Tukey test; Table 2). Different statistical results between shoot FW and DW results may explained by observed inability of BTH1 to hold its high shoot succulence (average values 75-78% of water in salinities below 171 mM NaCl) in salinities equal and higher than 513 mM NaCl (average values drop to 52-

59%). BTH2 plants showed higher global average succulence not statically affected by salinity (succulence ranged between 67% and 80%). This distinct behaviour for succulence was detected by a significant interaction genotype X salinity (Table 2).

Salicornia neei genotypes showed their largest average biomass at low salinity levels (BTH1 at 86 mM NaCl and BTH2 at 34 mM NaCl). Plants of BTH1 and BTH2 genotypes exposed to these low salinities showed relative biomass (RDW), respectively, of 129% and 119% of the biomass at the absence of salinity.

3.1.2. Pigments and photooxidative stress indices

The averages concentrations of photosynthetic pigments per shoot dry weight are shown in Table 3. All average concentrations of pigments in BTH1 genotype shoots were higher than observed in BTH2 shoots. Chlorophylls content were negatively affected by salinity in both *S. neei* genotypes. BTH1 had the highest averages of chlorophylls *a* (190 μg g^{-1}) and *b* (103 μg g^{-1}) in control treatment (0 mM NaCl) and lowest values in 513 mM NaCl (*a* = 78.0 μg g^{-1}; *b* = 40.4 μg g^{-1}), whereas highest and lowest averages of BTH2 genotype were observed in 34 mM NaCl (*a* = 152 μg g^{-1}; *b* = 67.1 μg g^{-1}) and 769 mM NaCl (*a* = 41.6 μg g^{-1}; *b* = 22.2 μg g^{-1}), respectively. *Salicornia neei* genotypes showed different chlorophyll *a*:*b* ratios (higher in BTH2; $F = 15.3$, $p < 0.001$) and distinct behaviour under salinity stress (significance interaction genotype X salinity; $F = 10.9$ and $p = 0.002$). BTH1 chlorophyll *a*:*b* ratio was not affected by salinity, whereas BTH2 values decreased with increasing salinities (Tukey test; Figure 1B, C).

Zeaxanthin content and de-epoxidation state of xanthophylls (DES) values were not detected in BTH2 genotype but both parameters were positively affected by salinity in BTH1 ($F = 16.4$, $p < 0.001$). BTH1 zeaxanthin averages ranged between 0.39 μg g^{-1} (at 34 mM NaCl) and 0.88 μg g^{-1} (at 86 mM NaCl), and de-epoxidation state (DES) between 0.09 ± 0.03 (0 mM NaCl) and 0.43 ± 0.09 (at 513 mM NaCl) (Figure 1A). The contents of other xanthophylls (lutein, neoxanthin and violaxanthin) in shoots of both genotypes were also reduced with increasing salinities (Table 3). Maximum xanthophyll contents were observed between 0 (BTH1) and 34 (BTH2) mM NaCl, and minimum

values between 513 (BTH1) and 769 (BTH2) mM NaCl. Salinity did not affect statically the β-carotene content in BTH1 shoots, although a tendency of reduction of the average concentration with the increase of the salinity. The highest β-carotene average value was observed at 0 mM NaCl (1.98 μg g^{-1}), as well as the content of this carotenoid in BTH2 shoots was detected only in two lower salinities (0 mM NaCl = 0.13 μg g^{-1}; 34 mM NaCl = 0.43 μg g^{-1}). The total chlorophyll to total carotenoids ratio was not affected by salinity in both *S. neei* genotypes, but BTH1 shoots had a higher ratio than BTH2 (Table 3).

3.1.3. Phenolic compounds and antioxidant activity

Shoots of BTH1 and BTH2 showed high and similar FPC contents (Table 4), not affected by salinity. FPC averages ranged from 11.5 to 15.7 mg GAE g^{-1} in BTH1, and from 12.3 to 13.9 mg GAE g^{-1} in BTH2. The concentrations of FFC, syringic acid and vanillin were higher in BTH1 shoots and quercetin content was higher in BTH2 shoots. FFC content was not affected by salinity. Salinity negatively affected the contents of vanillin and syringic acid (only BTH1 genotype). Contrariwise, quercetin content was positively affected by salinity increment and both genotypes showed lower averages at 86 mM NaCl (BTH1 = non detected; BTH2 = 5.45 μg g^{-1}) and higher values at 769 mM NaCl (BTH1 = 4.56 μg g^{-1}; BTH2 = 11.6 μg g^{-1}).

BTH1 shoot extract had higher antioxidant activity than BTH2, but IC_{50} was not affected by salinity. Higher antioxidant capacities (lower IC_{50} values) of BTH1 (IC_{50} = 4.37 mg mL^{-1}) and BTH2 (IC_{50} = 5.31 mg mL^{-1}) were observed at 86 and 171 mM NaCl, respectively. Antioxidant activity was correlated with only FPC ($r = -0.69$, $p < 0.05$) and FFC content ($r = -0.56$, $p < 0.05$) in BTH1 genotype. That is, the higher the content of FPC and FFC the lower the IC50 (higher antioxidant activity). For BTH2, antioxidant activity was not correlated with anything metabolites evaluated.

3.2. Growth, biomass production and shoot composition of S. neei genotypes under saline effluent irrigation at field conditions

Salicornia neei genotypes had initial values of height (BTH1 =19.8 ± 1.54 cm; BTH2 = 24.2 ± 1.71 cm), FW (BTH1 = 26.4 ± 0.35 g; BTH2 = 27.3 ± 0.38 g) and DW (BTH1 = 6.23 ± 0.99 g; BTH2 = 9.04 ± 1.10 g) significantly different. After 154 days irrigated with saline shrimp farm effluent in field, no plant death was observed. Contrasting with the results of greenhouse experiment, BTH2 genotype, in general, showed better performance than BTH1 (Table 5). Average values of FW, DW and succulence of BTH2 plants were higher than BTH1, resulting in superior BTH2 yield (5.74 FW ton. ha^{-1}; BTH1 = 2.05 FW ton. ha^{-1}). No statistical difference was found between genotypes for shoot height and branch number.

The average total contents of FPC (22.2 mg GAE g^{-1}) and FFC (5.06 mg QE g^{-1}) in BTH2 shoots were 2 and 10-folds higher than in BTH1 shoots, respectively (Table 5). Higher contents of phenolic and a larger number of phenolic acids were quantified in the field cultivated plants. BTH1 showed higher content of vanillin, syringic and ferulic acids, whereas BTH2 had higher concentration of quercetin, as well as for caffeic, chlorogenic, gallic and protocatechuic acids. Although it was necessary a 2.6-fold smaller amount of BTH2 shoot mass (IC_{50} = 5.41 mg mL^{-1}) than BTH1 mass (IC_{50} = 14.3 mg mL^{-1}) to inhibit 50% of DPPH radicals no statistical difference was found between the antioxidant activities of *S. neei* genotypes.

4. Discussion

4.1. Responses of S. neei genotypes to different salinity levels

The two new genotypes of *Salicornia neei* behave as constitutive halophytes, showing maximum growth under low salinities rather than in the absence of salt. This response, observed in other succulent extreme halophytes, results from a better homoeostasis under salt conditions (Rozema & Schat, 2013). Furthermore, several growth parameters (height, branch number and shoot DW) and biochemical characteristics (FPC, FFC and antioxidant activity) showed only minor and non-significant differences among the salinity levels tested. Our results point out that, in order to sustain growth under salt stress, *S. neei* genotypes regulate their physiological

mechanisms related to succulence, production of photoprotective pigments and metabolites able to scavenger oxidative radicals.

Maximum water content in the shoot biomass of *S. neei* genotypes cultivated in the greenhouse was 78-80%, in average. These values were slightly lower than wild plants of this species in Brazil (88.2–88.6%; Bertin et al., 2014) and field cultivated BTH1 and BTH2 plants (overall average of 85.8%; Doncato & Costa, 2017). High succulence of *S. neei* genotypes was observed between 0 and 171 mM NaCl; above this range succulence decreases for BTH1 and did not significatively changed for BTH2. Redondo-Gómez et al. (2006) and Garcia-Caparros et al. (2017) did not find variation in root and shoot water content of *Sarcocornia fruticosa* grown under greenhouse at salinity levels ranging between 0-1030 mM NaCl and 10-300 mM NaCl, respectively. . This response contrasts with the more common mechanism used by succulent halophytes against saline stress, which is water absorption for metabolic functions by salt ion compartmentation (e.g. Na^+ and Cl^-) into the vacuole, allowing maintenance of the osmotic and ionic balances between intra and extracellular media, including salt dilution in the vacuole (Rozema & Schat, 2013; Flowers & Colmer, 2015). The decrease of succulence in BTH1 genotype and the maintenance of succulence by BTH2 genotype at high salinities were both associated with visually detected epithelial lignification of their shoots. This lignification response was also observed by other authors (Flowers & Colmer, 2015; Moura, Bonine, Viana, Dornelas, & Mazzafera, 2010), which suggested that at toxic ionic vacuolar concentrations, some halophytes can reduce water loss by lignifying shoot tissue instead increase succulence.

Lignin content was not directly evaluated in this work but the phenolic acids data may be used as proxies for lignin metabolism. Syringic acid and vanillin are generated by lignin degradation in industrial plants (vanillin production) (Fache, Boutevin, & Caillol, 2016) and in experimental tests (insoluble bound forms with lignin and carbohydrates) (Pourali, Asghari, & Yoshida, 2010). Thus, the tissue content of these two compounds may direct related to lignin biosynthesis. As for shoot succulence, vanillin and syringic acid content decreased in *S. neei* shoots with the increment of salinity, showing lower values in salinities above than 171 mM NaCl (Tables 2 and 3). Thus, under salt stress *S. neei* (particularly BTH1 genotype) seems to consume phenolic

acids (vanillin and syringic acid) for lignin biosynthesis. Furthermore, the lignification is activated in *Salicornia europaea* L. shoots exposed to 200 mM NaCl (or over), and this process can be associated with the decrease of pigments' content (Fan et al., 2013).

The flavonoid quercetin may also play an important role in the succulence-lignification adjustment to salt stress of *S. neei*. In both genotypes studied quercetin content decrease from 0 to 86 mM NaCl. This behaviour change in plants exposed to solutions with more than 171 mM NaCl, and quercetin shoot content continuously increase till the highest salinity tested (769 mM NaCl). Quercetin and its derivates perform multiple functions in plants such as reduction of ROS formed (associated with stomata closure), inhibition of the auxin efflux facilitators, and they can be contributed in lignification (Agati, Azzarello, Pollastri, & Tattini, 2012; Taiz & Zeiger, 2002). Apparently, the low salinity quercetin content in *S. neei* (but particularly BHT2 variety) decreases in favour of plant growth (high height, fresh and dry biomass, and succulence). However, this initial physiological adjusts (e.g. succulence) cannot be maintain by plants exposed to 171 mM NaCl (or higher) salinities, being necessary quercetin production for the antioxidant action and to contribute in plant lignification.

Another response to salinity stress detected in both *S. neei* genotypes is the reduction of photosynthetic pigments, which can lead to inhibition of photosynthesis (Dubey, 2005). Costa et al. (2006) found chlorophyll *a* production in *S. neei* (sin. *Salicornia gaudichaudiana*) to be more affected by salinity than by UV-B radiation levels. Tiku (1976) observed too that chlorophyll *a* concentration of *Salicorina rubra* plants irrigated with 240 mM NaCl (14 NaCl g L^{-1}) culture solution was 72% smaller than in the absence of salt. In fact, succulence, lignification and changes in photosynthesis are adaptation mechanisms related to salt stress. Besides decreasing concentration of photosynthetic pigments, *S. neei* genotypes show chromatic adaptations against oxidative stress in photosystem induced by salt stress. BTH1 genotype seems to use its high carotenoids content to energy dissipation of the photosynthetic reaction centres (generate by no electrons fixation in CO_2 in Calvin cycle) (Taiz & Zeiger, 2002). It was evidenced by the maintenance of high ratio of these photoprotective pigments in relation to light-harvesting chlorophylls and the enhancement of de-epoxidation state (DES) of xanthophylls (Figure 1A) at increasing

salt concentration. Under strong salt stress, violaxanthin is de-epoxidized and transformed in zeaxanthin, which plays a direct antioxidant role, acting as a lipid protective (Latowski et al., 2011; Taiz & Zeiger, 2002). Previously, Costa et al. (2006) observed the elevation of zeaxanthin content in *Salicornia bigelovii* shoots exposed to increasing UV-B solar radiation and associated it with the protective effect of this compound against photooxidative damage of chloroplasts. According Duarte et al. (2013), the conversion of violaxanthin to zeaxanthin is one of the most effective energy dissipation mechanisms. These authors also found that *Sarcocornia fruticosa* photosynthetic rate decreases under salt stress but mainly due to both the reduction of the electron flow from the electron transport chain to the quinone pool and the quinone reduction turnover rate. These responses lead to an excess of energy accumulated at the level of cyclic photophosphorylation in photosystem I, where neither O_2 nor NADPH are produced. Previously, Redondo-Gómez et al. (2006) showed that net photosynthetic rate of *S. fruticosa* increased as the quantum efficiency of photosystem II decreased with the increment of salt stress, which means that the plant could be increasing photorespiration and/or using cyclic electron transport as additional photo-protective mechanisms. This perennial Salicornioideae species is close related with *S. neei* and further studies are necessary to verify if BTH1 genotype uses a similar biochemical response to salinity at the photosystem I level. Contrasting, BTH2 genotype mitigates photooxidative stress controlling the chlorophyll *a*:*b* ratio, which decrease due to the increment of chlorophyll b percentage in the total chlorophyll content along the salinity gradient (Figure 1C). Chlorophyll *b* has a protective function of photosystem due to the lower photo-oxidation rate than chlorophyll *a* (Dubey, 2005).

In general, biomass production in new genotypes of *S. neei* was not affected by salinity because of their distinct adaptive modulation for salt stress tolerance. Overall, the best salinity for BTH1 genotype was 86 mM NaCl, where it showed the highest growth values (averages of height, branch number, dry weight and succulence), contents of zeaxanthin, FPC and FFC, and antioxidant activity, as well as the lowest quercetin content. Differently, BTH2 did not show a clear best salinity growth level, whereas at 34 mM NaCl solution it showed highest values of biomass (fresh and dry), succulence, content of all photosynthetic pigments (except zeaxanthin that was not detected in any salinity level) and FPC, but lowest quercetin content. BTH1 genotype had higher but

much more salt sensitive contents of all metabolites studied (except quercetin), which felt abruptly at high salt concentrations. Although not statistically salt-affected, the antioxidant capacity of the BTH1 genotype was correlated with the content of phenolic compounds (FPC and FFC). This correlation was also observed by Costa et al., (2018) in the *S. neei* red biotype (plants that gave rise to the BTH1 genotype). Overall, BTH2 *S. neei* genotype was more growth efficient and salt tolerance than BTH1 genotype. The mechanism(s) of BTH2 for higher salt tolerance is not clear and may be associated with metabolic pathways not appreciated in the present study. For instance, Duarte et al. (2013) pointed out that in salt stressed plants of *Sarcocornia fruticosa* the enzyme superoxide dismutase (SOD) was the predominant form of enzymatic antioxidant defence.

4.2. Responses of S. neei genotypes under saline effluent irrigation at field conditions

The two new *S. neei* genotypes showed better growth performance and more succulent shoots in the field than during greenhouse experiment. Similar contrasting results were found with accessions of *Salicornia bigelovii* Torr. (Bresdin et al., 2016). Different performance of *S. neei* genotypes between experiments can be associated with availability of solar irradiation for plants. Inside the greenhouse, the average solar irradiation was 6.86 MJ m^{-2} day^{-1}, less than half of the available light in the field (19.8 MJ m^{-2} day^{-1}). Stressful environmental conditions (salinity plus high solar irradiation) favoured the performance of BTH2 genotype, which showed higher values of growth attributes than BTH1, contrariwise the greenhouse experiment where they showed similar heights and shoot biomass or even more branches in BTH1 shoots. These results corroborate the hypothesis of BTH2 *S. neei* genotype being more growth efficient and stress tolerant than BTH1.

Shoot heights reached by *S. neei* genotypes were higher than in field cultivation of previous years in the same local (Doncato & Costa, 2017) and of other sea asparagus species, for instance, *Salicornia persica* and *Salicornia fructicosa* (Ventura et al., 2011). Shoot biomass production of BTH2 *S. neei* (5.74 ton fw. ha^{-1}) irrigated with saline shrimp farm effluent was equivalent to values reached by vegetables irrigated with fresh

water, such as common asparagus (5.83 ton. FW ha^{-1}), however lower than of artichoke (12 ton. FW ha^{-1}), spinach (14.1 ton. FW ha^{-1}), and brassicas plants (broccoli and cauliflower; 16.4 ton. FW ha^{-1}) (FAO, 2018). Any all, it is important to highlight that other field trials showed that *S. neei* can achieve a shoot yield of 23 ton. FW ha^{-1} under irrigation with saline shrimp farm effluent after 3–4 months of cultivation (Costa et al., 2014).

Additionally, *S. neei* shoots showed a different phenolic acids profile and higher quercetin contents than plants cultivated in the greenhouse. Distinct environmental characteristics of these two cultivations should affect the tissue contents of phenolic compounds (Costa et al., 2006; Duarte et al., 2013). For instance, the greenhouse was covered with a 150 µm thick photostabilized LDPE Film with UV Absorber, and full UV exposition of *S. neei* in the field plot can explain the production of some phenolic compounds by sea asparagus plants (Costa et al., 2006). Field cultivated BTH2 plants had an average FPC content higher than in ethanolic and methanolic extracts of the vegetative shoots of green (respectively, 1.89 and 1.94 mg GAE g^{-1} dw; calculated considering 80% shoot water content) and red (respectively, 2.20 and 2.35 mg GAE g^{-1} dw) wild biotypes of *S. neei* from southern Brazil, cultivated with saline effluent of shrimp farm (Costa et al., 2018). Free phenolic compounds and FFC of BTH2 shoots were higher than found in methanolic extracts of wild plants of the *Sarcocornia perennis* from Tunisia (Gargouri et al., 2013) and *Salicornia europaea* collected in Tukey (Zengin et al., 2018), which range between 4.32-9.89 mg GAE g^{-1}dw and 3.3-5.1 mg catechin equivalent (ce) g^{-1} dw, respectively. The phenolic compounds in BTH2 shoots were also found in concentrations equal or greater than of organic asparagus (FPC = 19.4 mg GAE g^{-1}; FFC = 5.28 mg ce g^{-1} dw)(Ku et al., 2018), artichoke (FPC = 0.84 mg GAE g^{-1}; FFC = 0.65 mg ce g^{-1} dw), spinach (FPC = 0.6 mg GAE g^{-1}; FFC = 0.03 mg ce g^{-1} dw) (Alarcón-Flores, Romero-González, Vidal, & Frenich, 2014), broccoli and cauliflower (FPC ranged from 8.24 to 17.2 mg GAE g^{-1}) (dos Reis et al., 2015). Quercetin content of BTH2 shoots was higher than observed in artichoke and spinach (1.7 - 1.9 µg g^{-1}). Field cultivated BTH2 *S. neei* genotype demonstrated antioxidant capacity against DPPH radicals (IC_{50} = 5.41 mg mL^{-1}) higher than others sea asparagus cited by Barreira et al. (2017; *Sarcocornia perennis alpini* – IC_{50} = 11.5 mg mL^{-1}; *Sarcocornia perennis perennis* - IC_{50} = 8.04 mg mL^{-1}; *Salicornia ramosissima* -

IC_{50} = 5.69 mg mL^{-1}) and white cabbage (5.78 mg mL^{-1}), and turnip roots (6.92 mg mL^{-1}) (Aires et al., 2011).

The differences in antioxidant capacity between *S. neei* genotypes, observed in field cultivated plants, can be associated with the distinct composition and individual quantities of phenolic compounds produced by each genotype. High contents of carotenoids, syringic acid and vanillin play an important role against oxidative stress for BHT1 genotype, as demonstrate by modulation of these compounds under increasing salinities, whereas high production of flavonoids (FFC and quercetin), and protocatechuic and caffeic acids by BHT2 plants in the field plot seems to be determinant of a 2.6-folds higher antioxidant activity of their shoot extracts than those from BTH1 plants. Indeed, the FFC content of BTH2 shoot represents around 25% of all phenolic compounds (FPC), a much higher percentage than detected in BTH1 genotype (around 4% of FPC). Previously, Oh et al. (2007) found quercetin as the most abundant phenolic compound detected in ethanolic extracts of *Salicornia herbacea* shoots, as well as isolated quercetin and catechin acid were specially potent to scavenger DPPH radical. Phenylpropanoid acids, such as protocatechuic and caffeic acids that appear only in plants of *S. neei* cultivated in the field, are frequently concentrated in cuticles and cell walls exerting specific roles such as protection against oxidative damage, stabilizing reactive phenoxy radicals formed by intense UV radiation exposure (Shepherd, Macfarlane, & Colmer, 2005).

5. Conclusions

The present study revealed that BTH1 and BTH2 genotypes of *S. neei* have high tolerances to salt stress but distinct mechanisms of energy dissipation to avoid reactive oxygen species formation. BTH1 genotype maintain high ratio of photoprotective carotenoids in relation to light-harvesting chlorophylls and enhance the conversion of violaxanthin to zeaxanthin in order to dissipate exceeding energy photosystems at increasing salt concentration. BTH2 genotype mitigates photooxidative stress increasing the relative content of chlorophyll *b* in the photosynthetic pigments and by raising its shoot content of flavonoids (e.g. quercetin) for enhancement of antioxidant action. BTH2 genotype had higher growth, metabolites production and antioxidant capacity in

the field experiment irrigated with shrimp farm effluent than BTH1 genotype and also traditional crops irrigated with fresh water.

6. References

Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science*, *196*, 67–76. https://doi.org/10.1016/j.plantsci.2012.07.014

Aires, A., Fernandes, C., Carvalho, R., Bennett, R. N., Saavedra, M. J., & Rosa, E. A. S. (2011). Seasonal effects on bioactive compounds and antioxidant capacity of six economically important brassica vegetables. *Molecules*, *16*(8), 6816–6832. https://doi.org/10.3390/molecules16086816

Alarcón-Flores, M. I., Romero-González, R., Vidal, J. L. M., & Frenich, A. G. (2014). Determination of phenolic compounds in artichoke, garlic and spinach by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry. *Food Analytical Methods*, *7*(10), 2095–2106. https://doi.org/10.1007/s12161-014-9852-4

Barreira, L., Resek, E., Rodrigues, M. J., Rocha, M. I., Pereira, H., Bandarra, N., … Custódio, L. (2017). Halophytes: Gourmet food with nutritional health benefits? *Journal of Food Composition and Analysis*, *59*, 35–42. https://doi.org/10.1016/j.jfca.2017.02.003

Bertin, R. L., Gonzaga, L. V., Borges, G. da S. C., Azevedo, Mô. S., Maltez, H. F., Heller, M., … Fett, R. (2014). Nutrient composition and, identification/quantification of major phenolic compounds in *Sarcocornia ambigua* (Amaranthaceae) using HPLC-ESI-MS/MS. *Food Research International*, *55*, 404–411. https://doi.org/10.1016/j.foodres.2013.11.036

Bresdin, C., Glenn, E. P., & Brown, J. J. (2016). Comparison of Seed Production and Agronomic Traits of 20 Wild Accessions of *Salicornia bigelovii* Torr. Grown Under Greenhouse Conditions. In M. A. Khan, M. Ozturk, B. Gul, & M. Z. Ahmed (Eds.), *Halophytes for Food Security in Dry Lands* (pp. 67–82). Elsevier. https://doi.org/10.1016/B978-0-12-801854-5.00005-4

Cavalcanti, F. R., Lima, J. P. M. S., Ferreira-Silva, S. L., Viégas, R. A., & Silveira, J. A. G. (2007). Roots and leaves display contrasting oxidative response during salt stress and recovery in cowpea. *Journal of Plant Physiology*, *164*(5), 591–600. https://doi.org/10.1016/j.jplph.2006.03.004

Costa, C. S. B., Armstrong, R., Detres, Y., Koch, E. W., Bertiller, M., Beeskow, A., … San Roman, N. (2006). Effect of ultraviolet-B radiation on salt marsh vegetation: Trends of the genus *Salicornia* along the Americas. *Photochemistry and Photobiology*, *82*(4), 878–886. https://doi.org/10.1562/2005-10-30-RA-729

Costa, C. S. B., Chaves, F.C., Rombaldi, C.V., & Souza, C.R. (2018). Bioactive compounds and antioxidant activity of three biotypes of the sea asparagus *Sarcocornia ambigua* (Michx.) M.A.Alonso & M.B.Crespo: a halophytic crop for cultivation with shrimp farm effluent. *South Africa Journal of Botany*, 117, 95–100. https://doi.org/10.1016/j.sajb.2018.05.011

Costa, C. S. B., Kadereit, G., & Freitas, G. P. M. (*in press*). Molecular markers indicate the phylogenetic identity of southern Brazilian sea asparagus: First record of *Salicornia neei* Lag. in Brazil. *Rodriguésia*.

Costa, C. S. B., Vicenti, J. R. M., Morón-Villareyes, J. A., Caldas, S., Cardoso, L. V, Freitas, R. F., & D'Oca, M. G. M. (2014). Extraction and characterization of lipids from *Sarcocornia ambigua* meal: a halophyte biomass produced with shrimp farm effluent irrigation. *Anais da Academia Brasileira de Ciências*, *86*(2), 935–943. https://doi.org/10.1590/0001-3765201420130022

Doncato, K. B., & Costa, C. S. B. (2017). Growth and mineral composition of two lineages of the sea asparagus *Sarcocornia ambigua* irrigated with shrimp farm saline effluent. *Experimental Agriculture*, 1–18. https://doi.org/10.1017/S0014479717000096

dos Reis, L. C. R., de Oliveira, V. R., Hagen, M. E. K., Jablonski, A., FlÔres, S. H., & de Oliveira Rios, A. (2015). Carotenoids, flavonoids, chlorophylls, phenolic compounds and antioxidant activity in fresh and cooked broccoli (*Brassica oleracea* var. Avenger) and cauliflower (Brassica oleracea var. Alphina F1). *LWT - Food Science and Technology*, *63*(1), 177–183. https://doi.org/10.1016/j.lwt.2015.03.089

Duarte, B., Santos, D., Marques, J. C., & Caçador, I. (2013). Ecophysiological adaptations of two halophytes to salt stress: Photosynthesis, PS II photochemistry and anti-oxidant feedback - Implications for resilience in climate change. *Plant Physiology and Biochemistry*, *67*, 178–188. https://doi.org/10.1016/j.plaphy.2013.03.004

Dubey, R. (2005). Photosynthesis in plants under stressful conditions. In M. Pessarakli (Ed.), *Handbook of Photosynthesis* (2ª Edition, pp. 710–730). Boca Raton, FL: Taylor & Francis Group.

Fache, M., Boutevin, B., & Caillol, S. (2016). Vanillin Production from Lignin and Its Use as a Renewable Chemical. *ACS Sustainable Chemistry and Engineering*, *4*(1), 35–46. https://doi.org/10.1021/acssuschemeng.5b01344

Fan, P., Nie, L., Jiang, P., Feng, J., Lv, S., Chen, X., … Li, Y. (2013). Transcriptome analysis of *Salicornia europaea* under saline conditions revealed the adaptive primary metabolic pathways as early events to facilitate salt adaptation. *PLoS ONE*, *8*(11), 1–18. https://doi.org/10.1371/journal.pone.0080595

FAO. (2018). FAOSTAT. Retrieved April 1, 2018, from http://www.fao.org/faostat/en/#home

Flexas, J., & Medrano, H. (2002). Drought-inhibition of photosynthesis in C3 plants: Stomatal and non-stomatal limitations revisited. *Annals of Botany*, *89*(2), 183–189. https://doi.org/10.1093/aob/mcf027

Flowers, T. J., & Colmer, T. D. (2015). Plant salt tolerance: adaptations in halophytes. *Annals of Botany*, *115*(3), 327–331. https://doi.org/10.1093/aob/mcu267

Freitas, R. F., & Costa, C. S. B. (2014). Germination responses to salt stress of two intertidal populations of the perennial glasswort *Sarcocornia ambigua*. *Aquatic Botany*, *117*, 12–17. https://doi.org/10.1016/j.aquabot.2014.04.002

Gajula, D., Verghese, M., Boateng, J., Walker, L. T., Shackelfor, L., Mentreddy, S. R., & Cedric, S. (2009). Determination of Total Phenolics, Flavonoids and Antioxidant and Chemopreventive Potential of Basil (*Ocimum basilicum* L. and *Ocimum tenuiflorum* L.). *International Journal of Cancer Research*, *5*(4), 130–143. https://doi.org/10.3923/ijcr.2009.130.143

García-Caparrós, P., Llanderal, A., Pestana, M., Correia, P.J., & Lao, M.T. (2017). Nutritional and physiological responses of the dicotyledonous halophyte *Sarcocornia fruticosa* to salinity. Australian Journal of Botany, 2017, 65, 573–581. https://doi.org/10.1071/BT17100

Gargouri, M., Magné, C., Dauvergne, X., Ksouri, R., El Feki, A., Metges, M. A. G., & Talarmin, H. (2013). Cytoprotective and antioxidant effects of the edible halophyte *Sarcocornia perennis* L.(swampfire) against lead-induced toxicity in renal cells. *Ecotoxicology and environmental safety*, 95, 44-51. doi:10.1016/j.ecoenv.2013.05.011

Hasegawa, P. P. M., Bressan, R. A., Zhu, J.-K., & Bohnert, H. J. (2000). Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology*, *51*, 463–499. https://doi.org/10.1146/annurev.arplant.51.1.463

Joven, J., Micol, V., Segura-Carretero, A., Alonso-Villaverde, C., Menéndez, J. A., Aragonès, G., … Vázquez-Martín, A. (2014). Polyphenols and the Modulation of Gene Expression Pathways: Can We Eat Our Way Out of the Danger of Chronic Disease? *Critical Reviews in Food Science and Nutrition*, *54*(8), 985–1001. https://doi.org/10.1080/10408398.2011.621772

Ku, Y. G., Bae, J. H., Namiesnik, J., Barasch, D., Nemirovski, A., Katrich, E., & Gorinstein, S. (2018). Detection of Bioactive Compounds in Organically and Conventionally Grown Asparagus Spears. *Food Analytical Methods*, *11*(1), 309–318. https://doi.org/https://doi.org/10.1007/s12161-017-1074-0

Latowski, D., Kuczyńska, P., & Strzałka, K. (2011). Xanthophyll cycle – a mechanism protecting plants against oxidative stress. *Redox Report*, *16*(2), 78–90. https://doi.org/10.1179/174329211X13020951739938

Mendes, C. R., Cartaxana, P., & Brotas, V. (2007). HPLC determination of phytoplankton and microphytobenthos pigments: Comparing resolution and sensitivity of a C18 and a C8 method. *Limnology and Oceanography: Methods*, *5*(10), 363–370. https://doi.org/10.4319/lom.2007.5.363

Moura, J. C. M. S., Bonine, C. A. V., Viana, J. de O. F., Dornelas, M. C., & Mazzafera, P. (2010). Abiotic and biotic stresses and changes in the lignin content and composition in plants. *Journal of Integrative Plant Biology*, *52*(4), 360–376. https://doi.org/10.1111/j.1744-7909.2010.00892.x

Nicklisch, S. C. T., & Waite, J. H. (2014). Optimized DPPH assay in a detergent-based buffer system for measuring antioxidant activity of proteins. *MethodsX*, *1*, 233–238. https://doi.org/10.1016/j.mex.2014.10.004

Oh, J.-H., Kim, E.-O., Lee, S.-K., Woo, M.-H., & Choi, S.-W. (2007). Antioxidant activities of the ethanol extract of Hamcho (*Salicornia herbacea* L.) cake prepared by enzymatic treatment. *Food Science and Biotechnology*, *16*(1), 90–98.

Patel, S. (2016). Salicornia: Evaluating the halophytic extremophile as a food and a pharmaceutical candidate. *3 Biotech*, *6*(1), 1–10. https://doi.org/10.1007/s13205-016-0418-6

Piirainen, M., Liebisch, O., & Kadereit, G. (2017). Phylogeny, biogeography, systematics and taxonomy of Salicornioideae (Amaranthaceae/Chenopodiaceae) – a cosmopolitan, highly specialized hygrohalophyte lineage dating back to the oligocene. *Taxon*, *66*(1), 109–132. https://doi.org/10.12705/661.6

Pourali, O., Asghari, F. S., & Yoshida, H. (2010). Production of phenolic compounds from rice bran biomass under subcritical water conditions. *Chemical Engineering Journal*, *160*(1), 259–266. https://doi.org/10.1016/j.cej.2010.02.057

Rahman, M. M., Kim, M.-J., Kim, J.-H., Kim, S.-H., Go, H.-K., Kweon, M.-H., & Kim, D.-H. (2018). Desalted *Salicornia europaea* powder and its active constituent, trans -ferulic acid, exert anti-obesity effects by suppressing adipogenic-related factors. *Pharmaceutical Biology*, *56*(1), 183–191. https://doi.org/10.1080/13880209.2018.1436073

Redondo-Gómez, S., Wharmby, C., Castillo, J.M., Mateos-Naranjo, E., Luque,

C.J., de Cires, A., Luque, T., Davy, A.J., Figueroa, M.E. (2006). Growth and photosynthetic responses to salinity in an extreme halophyte, *Sarcocornia fruticosa*. Physiologia Plantarum 128, 116–124. https://doi.org/10.1111/j.1399-3054.2006.00719.x

Rozema, J., & Schat, H. (2013). Salt tolerance of halophytes, research questions reviewed in the perspective of saline agriculture. *Environmental and Experimental Botany*, *92*, 83–95. https://doi.org/10.1016/j.envexpbot.2012.08.004

Scaglioni, P. T., de Souza, T. D., Schmidt, C. G., & Badiale-Furlong, E. (2014). Availability of free and bound phenolic compounds in rice after hydrothermal treatment. *Journal of Cereal Science*, *60*(3), 526–532. https://doi.org/10.1016/j.jcs.2014.08.005

Shepherd, K. A., Macfarlane, T. D., & Colmer, T. D. (2005). Morphology, anatomy and histochemistry of salicornioideae (chenopodiaceae) fruits and seeds. *Annals of Botany*, *95*(6), 917–933. https://doi.org/10.1093/aob/mci101

Souza, M. M., da Silva, B., Costa, C. S. B., & Badiale-Furlong, E. (*in press*). Free phenolic compounds extraction from Brazilian halophytes, soybean and rice bran by ultrasound-assisted and orbital shaker methods. *Anais Da Academia Brasileira de Ciencias*.

Taiz, L., & Zeiger, E. (2002). *Plant Physiology*. *Annals of Botany* (3rd ed.). Sinauer Associates. https://doi.org/10.1104/pp.900074

Tiku, B. L. (1976). Effect of salinity on the photosynthesis of the halophyte *Salicornia rubra* and *Distichlis stricta*. *Physiologia Plantarum*, *37*(1), 23–28.

Ventura, Y., Eshel, A., Pasternak, D., & Sagi, M. (2015). The development of halophyte-based agriculture: Past and present. *Annals of Botany*, *115*(3), 529–540. https://doi.org/10.1093/aob/mcu173

Ventura, Y., Wuddineh, W. A., Myrzabayeva, M., Alikulov, Z., Khozin-Goldberg, I., Shpigel, M., … Sagi, M. (2011). Effect of seawater concentration on the productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia* halophytes as leafy vegetable crops. *Scientia Horticulturae*, *128*(3), 189–196. https://doi.org/10.1016/j.scienta.2011.02.001

Zengin, G., Aumeeruddy-Elalfi, Z., Mollica, A., Yilmaz, M. A., & Mahomoodally, M. F. (2018). In vitro and in silico perspectives on biological and phytochemical profile of three halophyte species: A source of innovative

phytopharmaceuticals from nature. *Phytomedicine*, 38, 35-44. doi:10.1016/j.phymed.2017.10.017

Table 1. Average (± standard deviation) conditions of effluent (salinity, pH, dissolved oxygen - DO, nitrate, total ammonium nitrogen - TAN and phosphate), soil (moisture and electrical conductivity - EC) and meteorological conditions (maximum and minimum temperatures, solar radiation, and accumulated rainfall) during the field experiment.

Effluent	
Salinity (mM NaCl)	213 ± 17.0
pH	8.65 ± 0.19
DO (mg L^{-1})	7.59 ± 0.58
Nitrate (mg L^{-1})	< 0.03
TAN (mg L^{-1})	0.15 ± 0.62
Phosphate (mg L^{-1})	0.30 ± 0.24
Soil	
Moisture (%)*	12.4 ± 5.4
EC (dS m^{-1})*	14.3 ± 5.6
*Meteorological***	
T max. (ºC)	24.5 ± 2.6
T min. (ºC)	23.3 ± 2.7
Radiation (MJ m^{-2} day^{-1})	19.8 ± 6.97
Rainfall (mm)	340

*The plot soil moisture and EC were determined by Doncato & Costa, (2017).

**Daily meteorological data were obtained from the INMET station (Brazilian National Institute of Meteorology) located on the FURG campus (32°04 43 S; 52°10 03 W), approximately 20 km from the field site.

Table 2. Averages (± standard deviation) of height (cm), branch number, fresh weight (g), dry weight (g), RDW (%) and succulence (%) of *Salicornia neei* genotypes (BTH1 and BTH2) after 50 days of cultivation in 0, 34, 86, 171, 513 and 769 mM NaCl in greenhouse experiment.

Genotype	Salinity	Height		Branch number		Fresh Weight		Dry Weight		RDW		Succulence	
BTH1	0	15.3 ± 1.7[a]		8.2 ± 5.6[ab]		0.60 ± 0.22[abc]		0.15 ± 0.05[a]		100		75.2 ± 3.3[ab]	
	34	13.6 ± 2.6[a]		5.6 ± 4.3[abc]		0.52 ± 0.11[abc]		0.11 ± 0.03[a]		75.7		78.3 ± 4.2[a]	
	86	15.4 ± 3.6[a]		8.8 ± 5.6[a]		0.85 ± 0.29[a]		0.19 ± 0.07[a]		129		77.8 ± 4.7[ab]	
	171	14.8 ± 2.2[a]		5.5 ± 3.6[abc]		0.43 ± 0.15[bc]		0.11 ± 0.03[a]		76.5		73.1 ± 6.0[abc]	
	513	13.8 ± 2.4[a]		5.8 ± 2.5[abc]		0.32 ± 0.14[bc]		0.13 ± 0.05[a]		88.1		59.1 ± 5.7[c]	
	769	14.3 ± 2.7[a]		6.9 ± 4.5[ab]		0.28 ± 0.18[c]		0.12 ± 0.04[a]		79.2		52.5 ± 15.1[c]	
BTH2	0	15.0 ± 3.6[a]		1.3 ± 1.7[c]		0.55 ± 0.23[abc]		0.11 ± 0.05[a]		100		79.5 ± 2.1[a]	
	34	15.6 ± 2.6[a]		3.2 ± 4.6[bc]		0.66 ± 0.29[ab]		0.13 ± 0.07[a]		119		80.2 ± 1.9[a]	
	86	16.1 ± 2.8[a]		2.7 ± 3.8[bc]		0.60 ± 0.20[abc]		0.13 ± 0.04[a]		116		78.1 ± 4.1[ab]	
	171	15.2 ± 2.5[a]		3.0 ± 3.6[abc]		0.50 ± 0.22[bc]		0.12 ± 0.03[a]		105		75.5 ± 5.1[ab]	
	513	15.3 ± 3.5[a]		3.8 ± 4.8[abc]		0.41 ± 0.30[bc]		0.11 ± 0.04[a]		103		66.9 ± 9.1[bc]	
	769	15.3 ± 2.7[a]		4.7 ± 4.0[abc]		0.55 ± 0.21[abc]		0.12 ± 0.03[a]		111		75.2 ± 8.6[ab]	
		F	*p value*	*F*	*p value*	*F*	*p value*	*F*	*p value*	-	-	*F*	*p value*
	Genotype	3.21	0.757	35.0	***< 0.001***	2.88	0.335	2.17	0.143	-	-	16.7	***< 0.001***
	Salinity	0.46	0.500	1.76	0.187	36.2	***< 0.001***	0.61	0.437	-	-	46.6	***< 0.001***
	Interaction	0.18	0.676	2.42	0.122	12.3	***0.016***	1.68	0.197	-	-	7.46	***0.007***

Tukey's HSD test results of interaction is presented by lowercase for each variable.

Table 3. Averages (± standard deviation) of photosynthetic pigment contents (in μg g^{-1}) of *Salicornia neei* genotypes (BTH1 and BTH2) after 50 days of cultivation inside a greenhouse at different salinities (0, 34, 86, 171, 513 and 769 mM NaCl).

Genotype	Salinity	Chlorophyll *a*	Chlorophyll *b*	β-Carotene[§]	Lutein	Neoxanthin	Violaxanthin	Zeaxanthin[§]	Σcar:Σchl
BTH1	0	190 ± 6.02[a]	103 ± 11.9[a]	1.98 ± 0.43[a]	28.6 ± 6.02[a]	7.20 ± 2.33[a]	4.46 ± 0.97[a]	0.44 ± 0.09[a]	0.14 ± 0.02[a]
	34	85.0 ± 8.26[abc]	46.7 ± 2.95[bc]	0.47 ± 0.05[a]	13.3 ± 0.49[abc]	2.98 ± 0.88[abc]	1.56 ± 0.13[b]	0.39 ± 0.10[a]	0.14 ± 0.01[a]
	86	155 ± 27.9[ab]	78.3 ± 14.6[ab]	0.99 ± 0.41[a]	20.0 ± 4.55[ab]	4.55 ± 1.65[ab]	2.29 ± 0.41[ab]	0.88 ± 0.51[a]	0.12 ± 0.01[a]
	171	103 ± 34.7[abc]	57.7 ± 20.7[abc]	0.76 ± 0.29[a]	14.4 ± 4.74[abc]	3.98 ± 1.13[abc]	1.76 ± 0.41[b]	0.57 ± 0.03[a]	0.14 ± 0.01[a]
	513	78.0 ± 33.1[bc]	40.4 ± 16.7[bc]	0.53 ± 0.17[a]	9.00 ± 2.74[bc]	2.13 ± 0.51[bc]	1.14 ± 0.35[bc]	0.82 ± 0.09[a]	0.12 ± 0.02[a]
	769	86.0 ± 22.5[abc]	45.6 ± 9.32[bc]	0.76 ± 0.15[a]	11.4 ± 2.14[abc]	2.54 ± 0.43[abc]	1.43 ± 0.72[c]	0.86 ± 0.24[a]	0.13 ± 0.02[a]
BTH2	0	87.3 ± 21.3[abc]	42.9 ± 12.5[bc]	0.13 ± 0.22	12.6 ± 3.92[abc]	2.93 ± 1.08[abc]	1.25 ± 0.25[b]	nd	0.13 ± 0.01[a]
	34	152 ± 5.70[ab]	67.1 ± 6.49[abc]	0.43 ± 0.37	17.2 ± 2.60[abc]	3.52 ± 1.51[abc]	1.28 ± 0.21[b]	nd	0.10 ± 0.02[a]
	86	66.5 ± 12.0[bc]	31.5 ± 4.46[bc]	nd	8.56 ± 1.01[bc]	2.13 ± 0.22[bc]	0.77 ± 0.22[bc]	nd	0.12 ± 0.01[a]
	171	61.8 ± 3.53[bc]	29.9 ± 0.62[bc]	nd	8.22 ± 0.58[bc]	1.94 ± 0.33[bc]	0.76 ± 0.19[bc]	nd	0.12 ± 0.01[a]
	513	41.9 ± 12.7[c]	23.2 ± 7.48[c]	nd	6.80 ± 3.21[c]	1.58 ± 0.99[c]	0.25 ± 0.22[cd]	nd	0.13 ± 0.03[a]
	769	41.6 ± 6.01[c]	22.2 ± 3.60[c]	nd	6.13 ± 1.00[bc]	1.54 ± 0.27[bc]	nd[d]	nd	0.12 ± 0.01[a]

	F	*p value*	*F*	*p value*	*F*	*p value*	*F*	*p value*	*F*	*p value*	*F*	*p value*	*F*	*p value*	*F*	*p value*
Genotype	11.0	***0.002***	18.0	***< 0.001***	-	-	18.3	***< 0.001***	16.9	***< 0.001***	63.6	***< 0.001***	-	-	6.46	***0.016***
Salinity	16.8	***< 0.001***	15.6	***< 0.001***	2.61	0.126	25.1	***< 0.001***	18.6	***< 0.001***	54.8	***< 0.001***	8.94	***0.009***	0.16	0.694
Interaction	0.01	0.936	0.25	0.624	-	-	0.04	0.840	0.03	0.854	7.00	***0.013***	-	-	1.91	0.177

Tukey's HSD test results of interaction is presented by lowercase for each variable. [§]Studied effect of salinity in BTH1 genotype because low concentration (nd - non detected) in great salinities in BTH2 genotype. Σcar:Σchl is the total chlorophyll to total carotenoids ratio.

Table 4. Averages (± standard deviation) of phenolic compounds` contents and antioxidant capacity of *Salicornia neei* genotypes (BTH1 and BTH2) after 50 days of cultivation inside a greenhouse at different salinities (0, 34, 86, 171, 513 and 769 mM NaCl).

Genotype	Salinity	FPC (mg GAE g^{-1})	FFC (mg QE g^{-1})	Quercetin (µg g^{-1})	Syringic acid (µg g^{-1})[§]	Vanillin (µg g^{-1})	IC50 (mg mL^{-1})
BTH1	0	14.8 ± 1.80[a]	0.67 ± 0.20[a]	3.82 ± 0.48[bcd]	1.44 ± 0.58[a]	5.87 ± 1.39[a]	4.78 ± 0.82[a]
	34	14.3 ± 1.04[a]	0.76 ± 0.06[a]	1.07 ± 1.81[cd]	1.48 ± 0.79[a]	5.22 ± 0.68[ab]	5.10 ± 1.32[a]
	86	15.7 ± 0.65[a]	0.82 ± 0.16[a]	nd[d]	1.11 ± 0.27[ab]	5.39 ± 0.94[ab]	4.37 ± 1.79[a]
	171	11.5 ± 2.64[a]	0.63 ± 0.15[a]	3.62 ± 3.11[bcd]	0.88 ± 0.39[ab]	4.78 ± 0.82[ab]	6.00 ± 0.59[a]
	513	11.7 ± 1.05[a]	0.50 ± 0.01[a]	4.36 ± 0.52[bcd]	0.35 ± 0.29[b]	4.09 ± 0.37[ab]	5.66 ± 0.30[a]
	769	14.3 ± 0.14[a]	0.59 ± 0.15[a]	4.56 ± 0.34[bcd]	0.23 ± 0.20[b]	4.15 ± 0.27[ab]	4.75 ± 0.54[a]
BTH2	0	13.7 ± 1.00[a]	0.43 ± 0.12[a]	6.43 ± 0.28[abcd]	0.23 ± 0.24	4.96 ± 0.70[ab]	5.88 ± 1.13[a]
	34	13.9 ± 0.96[a]	0.51 ± 0.08[a]	5.84 ± 0.83[abcd]	0.14 ± 0.14	4.33 ± 0.22[ab]	5.86 ± 1.17[a]
	86	12.4 ± 1.61[a]	0.40 ± 0.01[a]	5.45 ± 0.62[abcd]	nd	4.29 ± 0.10[ab]	6.65 ± 1.16[a]
	171	13.9 ± 0.94[a]	0.64 ± 0.23[a]	7.32 ± 4.85[abc]	nd	4.60 ± 1.16[ab]	5.31 ± 1.06[a]
	513	12.3 ± 1.04[a]	0.44 ± 0.19[a]	9.54 ± 2.73[ab]	nd	4.33 ± 0.27[ab]	6.50 ± 0.57[a]
	769	12.7 ± 1.20[a]	0.47 ± 0.12[a]	11.6 ± 5.19[a]	nd	3.54 ± 0.35[b]	5.51 ± 0.67[a]

	F	*p value*	*F*	*p value*	*F*	*p value*	*F*	*p value*	*F*	*p value*	*F*	*p value*
Genotype	1.09	0.305	13.8	***< 0.001***	36.5	***< 0.001***	-	-	6.26	***0.018***	5.95	***0.021***
Salinity	2.60	0.117	2.61	0.116	16.8	***< 0.001***	28.1	***< 0.001***	16.0	***< 0.001***	0.00	0.974
Interaction	0.01	0.929	2.02	0.164	1.78	0.192	-	-	1.05	0.313	0.12	0.730

Tukey's HSD test results of interaction is presented by lowercase for each variable. [§]Studied effect of salinity in BTH1 genotype because low concentration (nd - non detected) in great salinities in BTH2 genotype. FPC – Free Phenolic Compounds; FFC – Free Flavonoids Compounds; GAE – Gallic Acid Equivalent; QE – Quercetin Equivalent.

Table 5. Averages (± standard deviation) of growth parameters, phenolic compounds and antioxidant capacity of *Salicornia neei* genotypes (BTH1 and BTH2 after 154 days field growth irrigated with saline shrimp farm effluent.

			Student's t-Test	
	BTH1	BTH2	t	*p value*
Growth parameters				
Height (cm)	40.6 ± 6.81	51.6 ± 9.12	-2.16	0.065
Branch number	52.4 ± 11.8	52.8 ± 6.53	-0.07	0.949
Fresh Weight (g)	56.4 ± 10.2	158 ± 31.2	-6.92	***0.001***
Dry Weight (g)	14.9 ± 1.92	25.4 ± 5.40	-4.10	***0.009***
Succulence (%)	72.9 ± 5.97	84.0 ± 0.48	-4.30	***0.008***
Shoot yield (ton. FW ha^{-1})	2.05	5.74	-	**-**
Phenolic compounds				
FPC (mg GAE g^{-1})	13.1 ± 1.84	22.2 ± 2.38	-5.27	***0.007***
FFC (mg QE g^{-1})	0.54 ± 0.12	5.06 ± 1.83	-4.26	***0.005***
Quercetin (μg g^{-1})[‡]	nd	14.8[†]	-	-
Protocatechuic acid (μg g^{-1})	4.91 ± 0.06	10.0 ± 0.64	-11.2	***0.007***
Chlorogenic acid (μg g^{-1})	1.67 ± 1.01	3.46 ± 3.93	-0.75	0.519
Gallic acid (μg g^{-1})	0.38 ± 0.10	0.64 ± 0.24	-1.41	0.263
Caffeic acid (μg g^{-1})[‡]	nd	1.21 ± 0.43	-	-
Syringic acid (μg g^{-1})	6.82 ± 2.28	1.51 ± 0.37	3.25	***0.031***
Vanillin (μg g^{-1})	5.24 ± 0.78	2.73 ± 0.87	3.03	***0.039***
Ferulic acid (μg g^{-1})[‡]	0.39 ± 0.02	nd	-	-
Antioxidant activity				
IC_{50} (mg mL^{-1})	14.3 ± 7.11	5.41 ± 2.28	2.05	0.154

FPC – Free Phenolic Compounds; FFC – Free Flavonoids Compounds; GAE – Gallic Acid Equivalent; QE – Quercetin Equivalent; Nd - non detected. [†]Concentration was detected in one sample only; [‡] - Statistical analysis not performed.

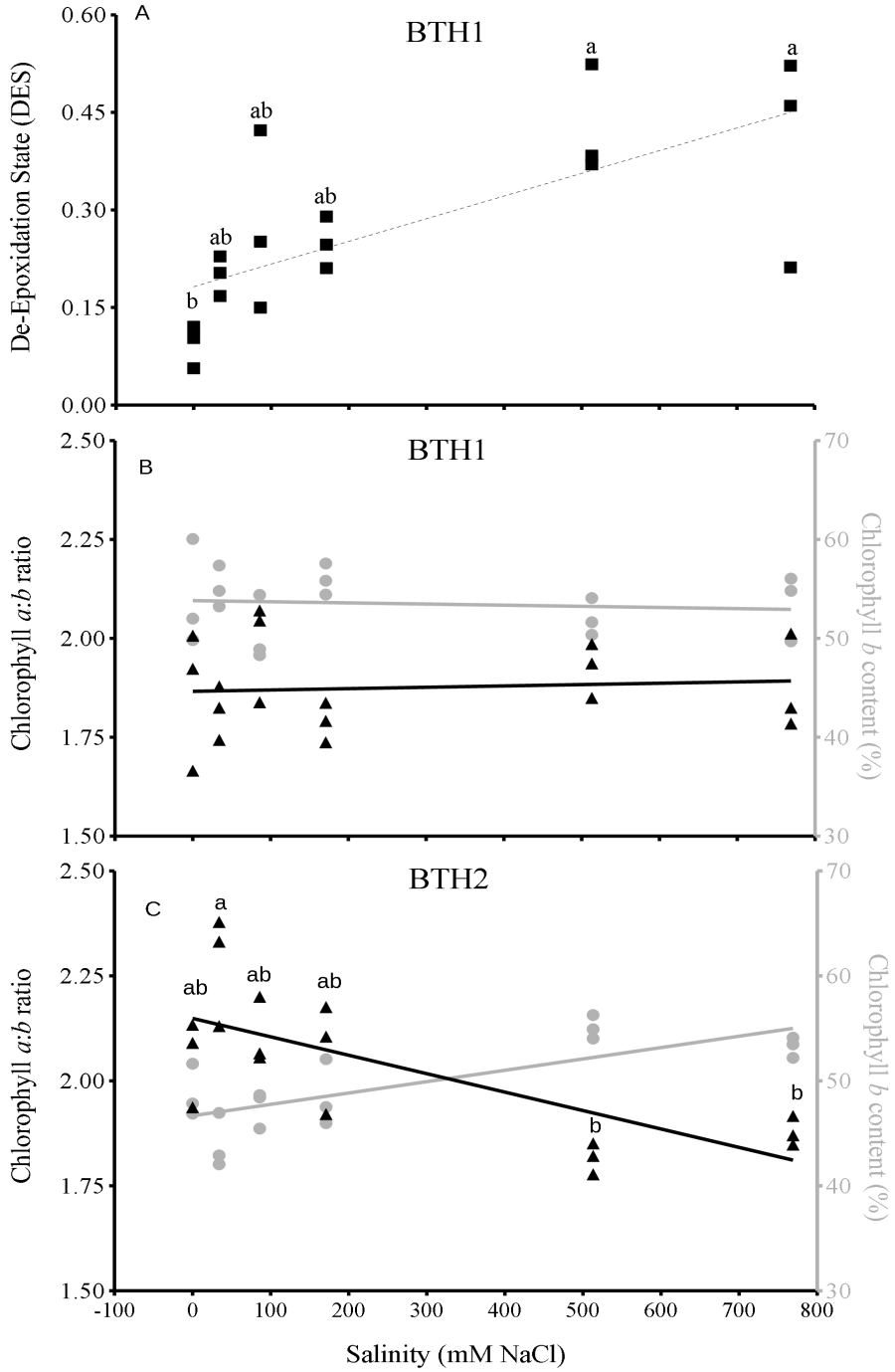


Figure 1. Photo-protective mechanisms of *Salicornia neei* genotypes under increasing salinities in a greenhouse experiment. The De-epoxidation state of xantophylls (DES) of the BTH2 genotype (A). Chlorophyll *a*:*b* ratio and content of chlorophyll b of the BTH1 (B) and BTH2 genotypes (C). Different lowercase letters represent significant differences among salinity levels.

Capítulo 4

Avaliação nutricional de diferentes níveis de farinha de *Salicornia neei* no crescimento e na vida útil da carne do camarão branco do pacífico (*Litopenaeus vannamei*)

RESUMO

O presente trabalho teve como objetivo avaliar o desempenho zootécnico e a vida útil da carne do camarão branco do pacífico *Litopenaeus vannamei* alimentado com diferentes níveis de inserção de farinha do aspargo marinho *Salicornia neei* na ração. Um total de 270 juvenis de *L. vannamei*, (1,29 ± 0,38 g) foram distribuídos aleatoriamente em nove tanques plásticos de 150 L (30 animais por tanque; 68,2 indivíduos m^{-2}) em sistema semi-estático. Os camarões foram alimentados com dietas contendo 0, 15 e 30% de farinha seca de *S. neei* por um período de 45 dias. Todos os tratamentos foram realizados em triplicata. Ao final do experimento foram estimados o ganho de peso, o ganho de peso semanal, o fator de conversão alimentar, as taxas de crescimento específico e de eficiência proteica, e a sobrevivência dos camarões. Além disso, 10 indivíduos de cada tanque foram armazenados em isopor com gelo (4 ºC) para avaliação da vida útil através das análises de cor, textura, pH, peroxidação lipídica e bases voláteis totais (BVT), as quais foram realizadas no 1º, 3º, 6º, 8º e 10º dias após o abate dos camarões. A sobrevivência foi de 100% em todos os tratamentos e os parâmetros de desempenho zootécnico não diferiram estatisticamente entre as dietas. O parâmetro de cromaticidade a* apresentou maiores valores com o aumento do nível de substituição. Já a cromaticidade b* tem uma tendência de aumentar ao longo do tempo, com o tratamento de 30% de substituição em geral apresentando os maiores valores. As concentrações de BVT apresentaram uma tendência de aumento ao longo do tempo, com diferenças significativas a partir do dia 8 e 10 nos tratamentos 15 e 30% de substituição, respectivamente. O tratamento com 30% de planta apresentou maiores conteúdos de BVT em relação ao controle no dia 10. O pH aumentou significativamente ao longo dos dias, porém sem diferir entre os tratamentos. A partir do dia 6, os valores de pH e conteúdo de BVT excederam os valores máximos preconizados, 7,85 e 30 mg N $100g^{-1}$, respectivamente, independente dos tratamentos. As dietas formuladas com *S. neei* apresentaram menores níveis de peroxidação lipídica nos dias 3, 8 e 10. Os camarões de todos os tratamentos avaliados apresentaram valores máximos de textura

no dia 6, com posterior redução. Como conclusão, a inserção de até 30% de *S. neei* reduz os níveis de peroxidação lipídica e pode causar alterações de coloração no músculo. Entretanto, não afeta o crescimento, a sobrevivência e a vida útil da carne de *L. vannamei*. Recomenda-se que o consumo da carne seja realizado em no máximo até 3 dias após o abate.

Palavras-chave: Halófita, Compostos funcionais, Dieta.

1. Introdução

Halófitas são plantas que habitam ambientes com alta concentração de sais (> 200 mM NaCl) e representam cerca de 1% da flora mundial (Flowers & Colmer, 2015; Norman et al., 2013). Nas últimas décadas o cultivo comercial de plantas halófitas vem crescendo devido a menor disponibilidade de água doce e aumento de áreas cultiváveis afetadas pela salinização, alcançando já no ano de 1990 uma produção entre 4 – 5 bilhões de toneladas com base nos dados da FAO (da sigla em inglês *Food and Agriculture Organization*) (Attia-Ismail, 2015; Flowers et al., 1977; Panta et al., 2014; Ventura et al., 2015). Ou seja, a produção de biomassa vegetal em áreas degradadas, onde não é possível plantar cultivares tradicionais, já é uma realidade e as plantas produzidas são consumidas pelo homem (Ventura et al., 2015) e animais como plantas forrageiras (Attia-Ismail, 2018) e são ingrediente de rações de ovelhas (Hintsa et al., 2018), cordeiros (Swingle et al., 1996), camelos (Mahmoud et al., 2016), peixes (Ríos-Durán et al., 2013) e camarões (Tomazelli Junior et al., 2017), sendo considerados uma boa fonte de nutrientes como proteínas, ácidos graxos, minerais, carboidratos e metabolitos secundários com propriedades bioativas (Barreira et al., 2017; Panta et al., 2014).

Inúmeros trabalhos mostram que plantas halófitas possuem propriedades bioativas tais como elevada capacidade antioxidante (Souza et al., *capítulo 2*), antimicrobiana (Essaidi et al., 2013), anti-helmíntico (Katiki et al., 2013) e antiviral (Tomazelli Junior et al., 2017). A inserção de farelo de folhas da halófita *Rhizophora mucronata* na ração do camarão branco do pacífico (*Litopenaeus vannamei*), por exemplo, além aumentar a sanidade e imunidade dos mesmos contra o vírus da doença da mancha branca (WSSV - do inglês *White Spot Syndrome Virus*), resultou em maiores sobrevivências (Chakraborty et al., 2014). O aporte nutricional e de compostos

bioativos poderia, além de nutrir e melhorar a sanidade, influenciar no desenvolvimento zootécnico destes animais. Chen et al. (2011), por exemplo, ao inserirem isoflavonas (um grupo de compostos fenólicos) na alimentação do camarão branco (*L. vannamei*) alcançaram um aumento do sistema imune dos animais e consequente aumento dan resistência ao *Vibrio alginolyticus*, resultando em melhor desempenho zootécnico (maior taxa de crescimento específico).

A inserção de halófitas como fonte nutricional barata e com compostos bioativos também pode influenciar a qualidade da carne do animal cultivado. A adição de óleos essenciais, ricos em compostos antioxidantes, na alimentação de truta arco-íris (*Oncorhynchs mykiss;* Ceppa et al., 2018) e anchova (*Pomatomus saltatrix;* Erkan et al., 2011), melhorou a qualidade e a vida útil dos filés, avaliado por meio dos níveis de peroxidação lipídica e coloração. O efeito positivo da suplementação de óleos essenciais também foi observado para camarões, cuja qualidade da carne também foi verificada (Citarasu, 2010). Segundo Badiale-Furlong (2018), a qualidade do alimento ofertado (*e.g.* com alto conteúdo de flavonoides) e as práticas de manejo durante o cultivo tem impacto direto na qualidade da carne produzida.

Apesar de todos os benefícios associados às halófitas, o seu fornecimento para alimentação animal deve ser efetuado com certa parcimônia. A presença de compostos antinutricionais, como oxalatos, nitrato, saponinas, taninos e cloreto de sódio, pode ser prejudicial a depender da concentração destes compostos e da espécie animal alimentada (Kumar, 1992; Mukherjee et al., 2011).

Aspargos marinhos são plantas dos gêneros *Salicornia* e *Sarcocornia* (Salicornioideae, Amaranthaceae) encontradas naturalmente em marismas e desertos salgados e já vem sendo cultivadas em diferentes países (*e.g.* Brasil, México, Estados Unidos da América, Portugal, Eritréia, Arábia Saudita e Israel) com águas salobra e/ou salinas (Bañuelos et al., 2018; Doncato and Costa, 2017; Fedoroff et al., 2010; Patel, 2016; Ventura et al., 2011). Apresentam alto valor nutricional (e.g. proteínas, ácidos graxos e minerais), elevado conteúdo de moléculas bioativas (*e.g.* compostos fenóis, flavonoides e carotenoides) (Barreira et al., 2017; Bertin et al., 2016, 2014; Costa et al., 2014; Doncato and Costa, 2017) e inúmeras atividades funcionais (e.g. antimicrobiana e antioxidante) (Essaidi et al., 2013; Patel, 2016), sendo parte da alimentação humana

(e.g. como salada verde ou conserva; Ventura et al., 2015) e para diversas outras espécies animais, como frangos de corte (Attia et al., 1997), camelos (*Camelus dromedarius*; (Mahmoud et al., 2016) e Tilápias do Nilo (*Oreocromis niloticus*; Belal and Al-Dosari, 1999; Ríos-Durán et al., 2013).

O Laboratório de Halófitos da Biotecnologia (BTH) da Universidade Federal do Rio Grande (FURG; Brasil), por meio de um programa de melhoramento gerou o genótipo BTH2 de *Salicornia neei* Lag. (anteriormente classificada como *Sarcocornia ambigua*; Piirainen et al., 2017) com singularidades genéticas, crescimento decumbente, cor verde na maturidade, elevada produtividade e elevado conteúdo mineral, compostos fenólicos (e.g. flavonoides e ácidos fenólicos), carotenoides e atividade antioxidante (Costa et al., *in press*; Doncato & Costa, 2017; Freitas and Costa, 2014; Souza et al., *capítulo 3*). Além disso, sua produção com efluentes salinos do cultivo de camarões e peixes já é uma realidade (Doncato & Costa, 2017; Oliveira, 2017).

Desta forma este trabalho tem como objetivo avaliar o desempenho zootécnico e a vida útil da carne de camarões branco do pacífico *Litopenaeus vannamei* alimentados com diferentes níveis de farinha do genótipo BTH2 de *Salicornia neei*.

2. Materiais e métodos

2.1. Obtenção da farinha de Salicornia neei

Mudas de *S. neei* BTH2 foram obtidas por propagação vegetativa (Costa & Herrera, 2016) de exemplares mantidos em estufa não climatizada no Laboratório de Biotecnologia de Halófitas (BTH), do IO - FURG. As mudas foram transferidas para bancadas hidropônicas de camada filtrante com circulação forçada (15 minutos ligada, 15 minutos desligada), com vazão aproximada de 385 L por hora. No sistema foi recirculado efluentes salinos de 513 mM oriundos do bijupirá (*Rachycentron canadum* Linnaeus, 1766) (Oliveira, 2017) e plantas entre 15 e 20 cm foram coletadas, lavadas com água destilada, secas em estufa (50 ºC) e cominuídas em moinha de facas. A composição proximal, determinada de acordo com (AOAC, 2000) e o conteúdo de compostos fenólicos totais livres e os flavonoides totais livres na farinha de *S. neei,*

determinados por espectrofotometria de acordo com Souza et al. (*em submissão 1 e 2*), são apresentados na Tabela 1.

2.2. Origem dos camarões e desenho experimental

Neste estudo foram utilizados juvenis de *Litopenaeus vannamei,* criados em sistema de bioflocos, obtidos do setor de berçário do Laboratório de Carcinocultura, Instituto de Oceanografia (IO), FURG. Durante o período de aclimatação (3 dias), os animais foram mantidos em tanque de polietileno de 3000 L em água do mar e alimentados com rações Potimar 40 J (40 % de proteína bruta) da Guabi® (Brasil).

O experimento foi realizado na Estação Marinha de Aquicultura (EMA, IO-FURG) em sistema semi-estático com tanques plásticos de 150 L de volume útil (área de fundo de 0,44 m²) e trocas diárias de 50% da água. Foi utilizado um fotoperíodo de 12:12 horas (claro:escuro) e aeração e aquecimento constantes. Trinta juvenis de *L. vannamei*, com peso médio de 1,29 ± 0,38 g (± desvio padrão), foram estocados numa densidade de 68,2 indivíduos m^{-2} em cada tanque. Durante 45 dias os camarões foram alimentados com dietas com 0, 15 e 30% de farinha seca de *S. neei*. Cada dieta foi replicada em três tanques distintos, com o total de 9 tanques. As rações foram ofertadas três vezes ao dia (8:00, 14:00 e 18:00 horas) seguindo as recomendações de Jory et al. (2001), ajustadas diariamente com base nas observações visuais das bandejas de alimentação. Restos de ração e fezes foram removidos diariamente por sifonamento para manutenção da qualidade da água. Após este procedimento, o volume de água retirado foi reposto nas mesmas condições experimentais. Ao final do experimento todos os camarões foram abatidos em banho de gelo, pesados (balança de precisão AD2000, Marte Científica, São Paulo, Brasil) e 10 indivíduos de cada tanque imediatamente armazenados em isopor com gelo na temperatura de 4 ºC para avaliação da vida útil.

Os dados de qualidade da água não diferiram estatisticamente entre os tratamentos, por isso são apresentadas como médias globais. Salinidade (28,5 ± 0,15) (HACH HQ40d, Loveland, Colorado, EUA), pH (7,95 ± 0,04) (pHmetro FE20-FiveEasyTM, Mettler Toledo, Brasil), temperatura (28,8 ± 0,29 ºC) e oxigênio dissolvido (6,42 ± 0,05 mg L^{-1}) (YSI 55, Yellow Springs, OH, EUA) foram monitorados

diariamente em cada unidade experimental. Nitrogênio amoniacal total (0,98 ± 0,16 mg L^{-1}) (UNESCO, 1983), nitrito (0,41 ± 0,07 mg L^{-1}) (Bendschneider & Robinson, 1952), nitrato (0,32 ± 0,01 mg L^{-1}) (Aminot & Chaussepied, 1983) e fosfato (0,27 ± 0,03 mg L^{-1}) (Murphy & Riley, 1962) foram mensurados semanalmente.

2.3. Preparo das dietas

Devido a composição proximal da farinha de *S. neei*, optou-se por inserir a halófita como fonte de extrativo não nitrogenado (ENN), não alterando as fontes proteicas das dietas. Todos os ingredientes utilizados para o preparo das rações foram peneirados (100 µm), pesados, misturados e processados em moedor de carne para formação de peletes, os quais foram secos em estufa (50 ºC) por 24 horas e armazenados em freezer (-18 ºC). As dietas foram formuladas para a obtenção de rações isoproteicas (35 g $100g^{-1}$) e isoenergéticas (8 g 100 g^{-1}). A composição proximal e o conteúdo de compostos fenólicos das dietas são apresentados na Tabela 2.

2.4. Parâmetros zootécnicos avaliados

Ao final do experimento os parâmetros de ganho de peso (GP, gramas), ganho de peso semanal (GPS, gramas $semana^{-1}$), fator de conversão alimentar (FCA), taxa de crescimento específico (TCE, % dia^{-1}) e taxa de eficiência proteica (TEP) foram calculados de acordo com as equações a seguir: GP = (peso final – peso inicial); GPS = [(peso final – peso inicial) / nº de semanas de duração do experimento]; FCA = (massa total de alimento fornecida / GP); TCE = {100 x [ln (peso final) – ln (peso inicial)] / dias de duração do experimento}; TEP = (GP / massa de proteína ingerida). A sobrevivência (%) foi determinada pela razão entre o número final e inicial de camarões vezes 100.

2.4. Medidas para avaliação da vida útil

A vida útil dos camarões foi avaliada por meio das análises de cor, textura, pH, peroxidação lipídica (por meio da análise de TBARS) e bases voláteis totais (BVT). As análises foram realizadas após 1, 3, 6, 8 e 10 dias do abate dos camarões. A cada dia de análise seis indivíduos de cada tratamento (dois de cada unidade experimental) foram removidos do gelo e o exoesqueleto e cefalotórax retirados. Após análise de cor e textura da carne, estes foram individualmente cominuídos, com auxílio de uma faca, e 3 pools forma preparados com dois camarões de cada tanque para as análises de pH, BVT e TBARS.

As análises de cor dos camarões foram realizadas em colorímetro Minolta® CR400, determinando o sistema de cor no espaço *L**a*b*. Os valores de L, a* e b* refletem a luminosidade (branco - valores positivos; preto – valores negativos), cromaticidade no espectro do vermelho (+) e verde (-), e cromaticidade no espectro amarelo (+) e azul (-), respectivamente. No início de cada dia de medição o equipamento foi calibrado utilizando um disco branco, iluminante D65, com uma temperatura de cor de 6504K e um índice de reprodução cromática de 95%.

A textura dos músculos dos camarões foi avaliada através da força máxima de cisalhamento, determinada por texturômetro (TA.XT plus *Texture Analyser*, Stable Micro System Inc.) com probe de lâmina de Warner Bratzler (HDP/BS) a 5 centímetros de altura. Antes de cada dia de análise o equipamento era calibrado com um peso padrão de 5 quilos. As análises de cor e textura foram sempre realizadas na parte inicial do pléon, próximo ao cefalotórax, no Laboratório de Tecnologia de Alimentos (LTA) da Escola de Química e Alimentos (EQA) da FURG.

O pH foi analisado em pHmetro de bancada após extração com água destilada. Em um grama de amostra foi adicionado 10 mL de água destilada, homogenizados com bastão de vidro e vórtex, e centrifugado a 3214 *g* por 10 minutos, e o pH foi determinado no sobrenadante.

Os níveis de peroxidação lipídica no músculo dos camarões foram determinados de acordo com Oakes & Van Der Kraak (2003). Este método quantifica substâncias reativas ao ácido tiobarbitúrico (TBARS), incluindo o malondialdeído (MDA), um produto final da peroxidação lipídica. As leituras de fluorescência das amostras

(excitação: 520 nm; emissão: 580 nm) foram realizadas em espectrofluorímetro (Biotek, Sinergy HT). Os resultados estão expressos em nmol TMP mg tecido úmido^{-1}, onde TMP corresponde ao tetrametoxipropano (ACROS Organics), o qual foi utilizado para estabelecimento da curva padrão.

A quantificação dos níveis de BVT foi realizada de acordo com metodologia adaptada de Okpala et al. (2014). A três gramas de amostra (carne) foi adicionado 12 mL de ácido tricloroacético 4%. Essa mistura foi centrifugada a 3000 g durante 3 minutos e filtrada em papel filtro. Cinco mL de NaOH 2 M foi adicionado a 5 mL do sobrenadante em tubos de destilação microkjedahl. O destilado foi coletado em erlenmeyer com 15 mL de HCl 0,01 M padronizado e destilado com NaOH 0,01 M padronizado.

2.5. Estatística

Todos os dados estão apresentados em média ± desvio padrão. A normalidade e homogeneidade dos dados foram verificadas pelos testes de Shapiro-Wilk e Levene, respectivamente. Por não atenderam os pressupostos de normalidade e/ou homocedasticidade, os dados de TCE e luminosidade (L) foram transformados pelo arcoseno $\{[\text{arcoseno } (TCE / 100) * (180 / \pi)]^{-2}\}$ e log(x), respectivamente. Os parâmetros de desempenho zootécnico foram analisados utilizando uma ANOVA de uma via. Os parâmetros de qualidade da carne foram analisados através de ANOVA de duas vias, considerando o tratamento e o tempo como fatores. Em ambos os casos, o teste *post hoc* de Tukey foi aplicado para determinar diferenças entre os grupos experimentais. Correlações de Spearman foram realizadas para verificar correlações entre os parâmetros de qualidade da carne (cor, textura, pH, TBARS e BVT). O nível mínimo de significância foi fixado em 5% ($p<0,05$) para todas as análises.

3. Resultados

Os parâmetros de desempenho zootécnico não apresentaram diferença estatística entre as dietas testadas e a taxa de sobrevivência foi de 100% durante o experimento (Tabela 3).

Quanto a vida útil dos camarões cultivados com diferentes dietas, todos os parâmetros apresentaram interação significativa entre tratamento (dieta) e tempo. Para luminosidade (*L*) foi observada uma média significativamente menor para animais tratados com a dieta com 30% de farinha de *S. neei* em relação ao controle (0%). O parâmetro de cromaticidade a* (no espectro do vermelho-verde) apresentou maiores valores com o aumento do nível de substituição, desde o primeiro dia de observação. A cromaticidade b* (no espectro do amarelo-azul) também apresentou maiores valores no tratamento de 30% de substituição, que aumentaram significativamente longo do tempo (Tabela 4). A tonalidade azul observada em todos os tratamentos está associada ao mimetismo do animal a coloração (preta) das unidades experimentais.

Foi observada uma tendência de aumento da peroxidação lipídica na carne dos camarões cultivados dentro do período avaliado. Os níveis de TBARS nos camarões submetidos as dietas formuladas com *S. neei* foram significativamente menores do que nos camarões do tratamento controle nos dias 3, 8 e 10 após o abate (Figura 1b).

O tratamento 30% de substituição à carne do camarão apresentou maiores conteúdos de BVT em relação ao controle (Figura 1a) apenas no dia 10 após o abate. As concentrações de BVT apresentaram aumento ao longo do tempo, porém diferenças estatísticas só foram detectadas a partir do dia 8 e 10, respectivamente, nos tratamentos 15% e 30% de substituição. O conteúdo de BVT foi positivamente correlacionado com o pH (correlação de Spearman; $R^2 = 0,71$, $p < 0,05$). O pH apresentou aumentos estatisticamente significativos com o passar dos dias em todos os tratamentos experimentais, os quais não diferiram entre si (Figura 1c). No dia 1 o tratamento com 30% de substituição apresentou textura significativamente menor que o controle (Figura 1d). Os camarões de todos os tratamentos avaliados apresentaram valores máximos de textura no dia 6, com redução nos dias subsequentes.

4. Discussão

A inserção do aspargo marinho *S. neei* na dieta do *L. vannamei* em até 30% não resultou em nenhum efeito negativo significativo nos parâmetros de desempenho zootécnico e na sobrevivência do camarão. Este é o primeiro trabalho que avalia a inserção de uma farinha de aspargo marinho na alimentação do *L. vannamei*. Anteriormente havia sido demonstrado a aceitabilidade de inserção de elevados conteúdos do aspargo marinho *Salicornia bigelovii* na dieta da Tilápia do Nilo (*Oreochromis niloticus*), tanto na forma de farelo de caules (até 40%da dieta) (Belal and Al-Dosari, 1999) como de farelo de sementes (autoclavadas; até 50% de sementes da dieta com adição de 2% de colesterol) (Ríos-Durán et al., 2013). Estes resultados sugerem que as biomassas dos aspargos marinhos têm baixa concentração de compostos antinutricionais, e que o elevado teor de matéria mineral dessas biomassas (diretamente relacionado aos porcentuais de inserção de *S. neei* na dieta) não afeta o desempenho zootécnico do camarão *L. vannamei*.

A inserção de farinha de *S. neei* na dieta do *L. vannamei* foi avaliada também quanto à qualidade dos camarões cultivados. A carne dos camarões alimentado com dietas formuladas com *S. neei* apresentou menor peroxidação lipídica (cerca de 30% menor em ambas as dietas com *S. neei*) do que os alimentados com a dieta controle. A análise de TBARS quantifica os produtos finais da peroxidação lipídica, uma reação em cadeia iniciada pela ação de um radical hidroxil que leva à degradação oxidativa de ácidos graxos poli-insaturados (PUFA) e consequente diminuição da fluidez da membrana, afetando vários processos biológicos (Halliwell & Gutteridge, 2007). A menor peroxidação lipídica da carne dos camarões alimentados com *S. neei* pode estar associada à mudança do perfil de ácidos graxos das dietas ofertadas. Na composição das dietas a inserção de percentuais de farelo de *S. neei* foi realizada através da redução dos percentuais de óleo de peixe, farelo de trigo e amido de milho. Óleos de peixe são ricos em PUFAs, como os ácidos eicosapentaenoico (EPA) e docosaexaenoico (DHA) (Lin & Huang, 2007). Diferentemente da *S. neei*, que apresenta cerca de 50% de ácidos graxos saturados (Costa et al., 2014). Desta forma, quanto maior o nível de inserção de farinha de *S. neei* na dieta menor concentração de PUFAs, resultando em menor peroxidação lipídica uma vez que quanto maior a insaturação do ácido graxo maior susceptibilidade a oxidação (Lin & Huang, 2007). Lim et al. (1997) mostraram que o padrão de ácidos graxos na carne de *L. vannamei* pode refletir a composição lipídica de sua dieta,

entretanto isto foi mais frequente quando os ácidos graxos presentes na ração continham um alto grau de insaturação. Menor peroxidação lipídica também foi observado em camarões *Fenneropenaeus indicus* alimentados com dietas preparadas com substituições de 50% do óleo de peixe por óleos vegetais (canola, soja e linhaça; ricos em insaturados) (Ouraji et al., 2009).

Outra hipótese para explicar os menores níveis de peroxidação lipídica nos tratamentos com planta é a elevada capacidade antioxidante do genótipo BTH2 da *S. neei*, já determinada em testes *in vitro* (Souza et al., *capítulo 3*). Dietas com maior teor de farinha de *S. neei* apresentaram maior conteúdo de compostos fenólicos toais e flavonoides (Tabela 2). Devido a sua capacidade antioxidante (Quideau et al., 2011), esses compostos podem minimizar a ação de radicais oxidativos, diminuindo a peroxidação dos lipídeos presentes na carne. Inibição da peroxidação lipídica também foi observada em carnes de camarões *L. vannamei* imersos em soluções de catequina (flavonoide).

De uma maneira geral, a textura, o pH e o conteúdo de BVT não diferiram entre as dietas ao longo dos dias, diferentemente do observado para os níveis de peroxidação lipídica. Essa diferença entre a degradação de proteínas e lipídeos pode estar à composição das dietas, uma vez que as principais fontes de proteínas das mesmas não foram alteradas. O processo de degradação das proteínas libera inúmeros compostos nitrogenados de característica básica (*e.g.* trimetilamina e amônia), que promovem a elevação do pH muscular. Significativa correlação entre pH e BVT também foi determinada em camarões *Penaeus japonicus* (López-Caballero et al., 2000) e *Macrobrachium rosenbergii* (Kirschnik & Viegas, 2004) estocados em gelo. Além de alterar o pH, a degradação de proteínas no músculo também afeta a textura do mesmo. O aumento inicial da textura da carne está associada à perda de água livre, que corresponde a cerca de 75% do tecido muscular de pescados (Fennema, 2000). Posteriormente a atuação das enzimas e micro-organismos tendeu a desestruturar as fibras musculares, liberando a água e diminuindo a firmeza da carne.

A carne dos camarões alimentados com *S. neei* (tratamentos 15 e 30%) apresentaram uma coloração tendendo ao vermelho (a* positivo) e ao amarelo (b* positivo), sendo mais evidente essa variação na dieta com 30% de farinha de halófita. A

mudança da cromaticidade pode estar associada ao elevado conteúdo de pigmentos presentes no genótipo BTH2 da *S. neei*, como clorofila (valor médio de 113 µg g^{-1}) e carotenoides (valor médio de 12,9 µg g^{-1}) (Souza et al., *capítulo 3*). A cromaticidade b* também aumentou significativamente ao longo dos dias de análise para todos os tratamentos avaliados e esta resposta pode estar associado ao aumento dos níveis de peroxidação lipídica (Xia et al., 2009).

Com base nos limites preconizados por lei e literatura para os níveis de BVT (limite máximo permitido = 30 mg N $100g^{-1}$) (Brasil, 2017), TBARS (3,0 mg MDA kg^{-1}) (Cadun et al., 2005) e pH (7,85) (Brasil, 2017) em crustáceos, os camarões *L. vannamei* cultivados com diferentes níveis de farinha de *Salicornia neei* na dieta não apresentaram extensão na vida útil da carne, estando aptos para o consumo humano até o 3º dia após o abate.

5. Conclusões

Neste trabalho ficou evidenciado que é possível inserir até 30% de farinha do genótipo BTH2 da *Salicornia neei* nas dietas do camarão marinho *Litopenaeus vannamei*, sem efeito no desenvolvimento zootécnico. O camarão alimentado com *S. neei* também apresentou alteração da coloração e diminuição dos níveis de peroxidação lipídica. Estas alterações devem ser avaliadas do ponto de vista mercadológico, bem como é recomendável que testes de degustação avaliem possíveis alterações no sabor da carne produzidas com a dieta com a halófita. Os camarões *L. vannamei* cultivados apresentaram boa qualidade para consumo humano até o 3º dia após o abate, independente da dieta ofertada.

6. Bibliografia

Aminot, A., Chaussepied, M., 1983. Manuel des analyses en milieu marin. Centre National pou l'Exploitation de Océans (CNEXO).

AOAC, A. of O.A.C., 2000. Official Methods of Analysis., 17 th. ed. AOAC International, Gaithersburg, Maryland.

Attia-Ismail, S., 2015. Nutritional and Feed Value of Halophytes and Salt Tolerant Plants, in: El Shaer, H.M., Squires, V.R. (Eds.), Halophytic and Salt-Tolerant Feedstuffs. CRC Press, pp. 106–126. doi:10.1201/b19862-10

Attia-Ismail, S.A., 2018. Halophytes as forages, in: Edvan, R.L. (Ed.), New Perspectives in Forage Crops. IntechOpen, pp. 69–87. doi:http://dx.doi.org/10.5772/intechopen.69616

Attia, F.M., Alsobayel, A.A., Kriadees, M.S., Al-Saiady, M.Y., Bayoumi, M.S., 1997. Nutrient composition and feeding value of *Salicornia bigelovii* torr meal in broiler diets. Anim. Feed Sci. Technol. 65, 257–263. doi:10.1016/S0377-8401(96)01074-7

Badiale-Furlong, E., 2018. Transformações bioquímicas dos tecidos animais empregados na alimentação humana. Capítulo 12 In: Lajolo, F. M. & Mercadante, A. Z. (Eds.), Química e Bioquímica dos Alimentos. Coleção ciência, tecnologia, engenharia de alimentos e nutrição (CCTEAN), Volume 2. Ed. Atheneu. São Paulo.

Bañuelos, J.A., Velázquez-Hernández, I., Guerra-Balcázar, M., Arjona, N., 2018. Production, characterization and evaluation of the energetic capability of bioethanol from *Salicornia Bigelovii* as a renewable energy source. Renew. Energy 123, 125–134. doi:10.1016/j.renene.2018.02.031

Barreira, L., Resek, E., Rodrigues, M.J., Rocha, M.I., Pereira, H., Bandarra, N., da Silva, M.M., Varela, J., Custódio, L., 2017. Halophytes: Gourmet food with nutritional health benefits? J. Food Compos. Anal. 59, 35–42. doi:10.1016/j.jfca.2017.02.003

Belal, I.E.H., Al-Dosari, M., 1999. Replacement of Fish Meal with Salicornia Meal in Feeds for Nile Tilapia *Oreochromis niloticus*. J. World Aquac. Soc. 30, 285–289. doi:10.1111/j.1749-7345.1999.tb00877.x

Bendschneider, K., Robinson, R.J., 1952. A new spectrophotometric method for the determination of nitrite in sea water. J. Mar. Res. 11, 87–96.

Bertin, R.L., Gonzaga, L.V., Borges, G. da S.C., Azevedo, Mô.S., Maltez, H.F., Heller, M., Micke, G.A., Tavares, L.B.B., Fett, R., 2014. Nutrient composition and, identification/quantification of major phenolic compounds in *Sarcocornia ambigua* (Amaranthaceae) using HPLC-ESI-MS/MS. Food Res. Int. 55, 404–411. doi:10.1016/j.foodres.2013.11.036

Bertin, R.L., Maltez, H.F., Gois, J.S. de, Borges, D.L.G.G., Borges, G. da S.C., Gonzaga, L.V., Fett, R., 2016. Mineral composition and bioaccessibility in *Sarcocornia ambigua* using ICP-MS. J. Food Compos. Anal. 47, 45–51. doi:10.1016/j.jfca.2015.12.009

Brasil, 2017. Decreto de lei N°9013 de 29 de Março de 2017 - Regulamenta a Lei nº 1.283, de 18 de dezembro de 1950, e a Lei nº 7.889, de 23 de novembro de 1989, que dispõem sobre a inspeção industrial e sanitária de produtos de origem animal. Brasília, DF, Brazil.

Cadun, A., Cakli, S., Kisla, D., 2005. A study of marination of deepwater pink shrimp (*Parapenaeus longirostris*, Lucas, 1846) and its shelf life. Food Chem. 90, 53–59. doi:10.1016/j.foodchem.2004.03.024

Ceppa, F., Faccenda, F., De Filippo, C., Albanese, D., Pindo, M., Martelli, R., Marconi, P., Lunelli, F., Fava, F., Parisi, G., 2018. Influence of essential oils in diet and life-stage on gut microbiota and fillet quality of rainbow trout (*Oncorhynchus mykiss*). Int. J. Food Sci. Nutr. 69, 318–333. doi:10.1080/09637486.2017.1370699

Chakraborty, S., Ghosh, U., Balasubramanian, T., Das, P., 2014. Screening, isolation and optimization of anti–white spot syndrome virus drug derived from terrestrial plants. Asian Pac. J. Trop. Biomed. 4, S107–S117. doi:10.12980/APJTB.4.2014C1042

Chen, X.R., Tan, B.P., Mai, K.S., Zhang, W.B., Wang, X.J., Ai, Q.H., Xu, W., Liufu, Z.G., Ma, H.M., 2011. Dietary administration of soybean isoflavones enhances the immunity of white shrimp Litopenaeus vannamei and its resistance against *Vibrio alginolyticus*. Aquac. Nutr. 17, 24–32. doi:10.1111/j.1365-2095.2009.00702.x

Citarasu, T., 2010. Herbal biomedicines: A new opportunity for aquaculture industry. Aquac. Int. 18, 403–414. doi:10.1007/s10499-009-9253-7

Costa, C.S.B., Herrera, O.B., 2016. Halophytic Life in Brazilian Salt Flats: Biodiversity, Uses and Threats, in: Khan, M.A., Boër, B., Ozturk, M., Clüsener-Godt, M., Gul, B., Breckle, S.-W. (Eds.), Sabkha Ecosystem V : The Americas. Springer International Publishing, Berlin, pp. 11–27. doi:10.1007/978-3-319-27093-7_2

Costa, C.S.B., Kadereit, G., Freitas, G.P.M., n.d. Molecular markers indicate the phylogenetic identity of southern Brazilian sea asparagus: First record of *Salicornia neei* Lag. in Brazil. Rodriguésia.

Costa, C.S.B., Vicenti, J.R.M., Morón-Villareyes, J.A., Caldas, S., Cardoso, L. V, Freitas, R.F., D'Oca, M.G.M., 2014. Extraction and characterization of lipids from *Sarcocornia ambigua* meal: a halophyte biomass produced with shrimp farm effluent irrigation. An. Acad. Bras. Cienc. 86, 935–943. doi:10.1590/0001-3765201420130022

Doncato, K.B., Costa, C.S.B., 2017. Growth and mineral composition of two lineages of the sea asparagus *Sarcocornia ambigua* irrigated with shrimp farm saline effluent. Exp. Agric. 1–18. doi:10.1017/S0014479717000096

Erkan, N., Tosun, Ş.Y., Ulusoy, Ş., Üretener, G., 2011. The use of thyme and laurel essential oil treatments to extend the shelf life of bluefish (*Pomatomus saltatrix*) during storage in ice. J. fur Verbraucherschutz und Leb. 6, 39–48. doi:10.1007/s00003-010-0587-x

Essaidi, I., Brahmi, Z., Snoussi, A., Ben Haj Koubaier, H., Casabianca, H., Abe, N., El Omri, A., Chaabouni, M.M., Bouzouita, N., 2013. Phytochemical investigation of Tunisian *Salicornia herbacea* L., antioxidant, antimicrobial and cytochrome P450 (CYPs) inhibitory activities of its methanol extract. Food Control 32, 125–133. doi:10.1016/j.foodcont.2012.11.006

Fedoroff, N. V., Battisti, D.S., Beachy, R.N., Cooper, P.J.M., Fischhoff, D.A., Hodges, C.N., Knauf, V.C., Lobell, D., Mazur, B.J., Molden, D., Reynolds, M.P., Ronald, P.C., Rosegrant, M.W., Sanchez, P.A., Vonshak, A., Zhu, J.-K., 2010. Radically Rethinking Agriculture for the 21st Century. Science (80-.). 327, 833–834. doi:10.1126/science.1186834

Fennema, O.R. (Ed.), 2000. Quimica de los alimentos, 2ª. ed. Acrbia, Zaragoza, Espanha.

Flowers, T.J., Colmer, T.D., 2015. Plant salt tolerance: adaptations in halophytes. Ann. Bot. 115, 327–331. doi:10.1093/aob/mcu267

Flowers, T.J., Troke, P.F., Yeo, A.R., 1977. The mechanism of salt tolerance in halophytes. Annu. Rev. Plant Physiol. 28, 89–121. doi:10.1146/annurev.pp.28.060177.000513

Freitas, R.F., Costa, C.S.B., 2014. Germination responses to salt stress of two intertidal populations of the perennial glasswort *Sarcocornia ambigua*. Aquat. Bot. 117, 12–17. doi:10.1016/j.aquabot.2014.04.002

Halliwell, B., Gutteridge, J.M.C., 2007. Free radicals in biology and medicine, Fourth. ed. Oxford University Press, New York, USA.

Hintsa, K., Berhe, A., Balehegn, M., Berhe, K., 2018. Effect of replacing concentrate feed with leaves of Oldman saltbush (Atriplex nummularia) on feed intake, weight gain, and carcass parameters of highland sheep fed on wheat straw in northern Ethiopia. Trop. Anim. Health Prod. 1–6. doi:https://doi.org/10.1007/s11250-018-1577-8

Jory, D.E., Cabrera, T.R., Dugger, D.M., Fegan, D., Lee, P.G., Lawrence, A.L., Jackson, C.J., Mcintosh, R.P., Castañeda, J., 2001. A global review of shrimp feed management: Status and perspectives, in: Browdy, C.L., Jory, D.E. (Eds.), The New Wave, Proceedings of the Special Session on Sustainable Shrimp Culture. THe World aquaculture Society, Baton Rouge, LA USA, pp. 104–152.

Katiki, L.M., Ferreira, J.F.S., Gonzalez, J.M., Zajac, A.M., Lindsay, D.S., Chagas, A.C.S., Amarante, A.F.T., 2013. Anthelmintic effect of plant extracts containing

condensed and hydrolyzable tannins on Caenorhabditis elegans, and their antioxidant capacity. Vet. Parasitol. 192, 218–227. doi:10.1016/j.vetpar.2012.09.030

Kirschnik, P.G., Viegas, E.M.M., 2004. Alterações na qualidade do camarão de água doce *Macrobrachium rosenbergii* durante estocagem em gelo. Ciência e Tecnol. Aliment. 24, 407–412. doi:10.1590/S0101-20612004000300019

Kumar, R., 1992. Anti-nutritional factors, the potential risk of toxicity and methods to alleviate them, in: Séedly, A., Publiese, P.-L. (Eds.), Leguminosae Trees and Other Fodder Tress as Protein Sources for Livestock. FAO, Rome, pp. 145–160.

Lin, W.Y., Huang, C.H., 2007. Fatty acid composition and lipid peroxidation of soft-shelled turtle, *Pelodiscus sinensis*, fed different dietary lipid sources. Comp. Biochem. Physiol. - C Toxicol. Pharmacol. 144, 327–333. doi:10.1016/j.cbpc.2006.10.006

Lim, C., Ako, H., Brown, C. L., & Hahn, K. (1997). Growth response and fatty acid composition of juvenile Penaeus vannamei fed different sources of dietary lipid. Aquaculture, 151(1-4), 143-153. doi:10.1016/S0044-8486(96)01500-1

López-Caballero, M.E., Pérez-Mateos, M., Borderías, J.A., Montero, P., 2000. Extension of the shelf life of prawns (*Penaeus japonicus*) by vacuum packaging and high-pressure treatment. J. Food Prot. 63, 1381–1388. doi:10.4315/0362-028X-63.10.1381

Mahmoud, A.H., Basmaeil, S.M., El-Shaikh, Y., Swelum, A.A., Abouheif, M.A., 2016. Effect of halophyte salicornia bigelovii torr and graded levels of dietary crude protein on feed performance and carcass traits of camels. Rev. Bras. Zootec. 45, 704–708. doi:10.1590/S1806-92902016001100009

Mukherjee, P.K., Ponnusankar, S., Pandit, S., Hazam, P.K., Ahmmed, M., Mukherjee, K., 2011. Botanicals as medicinal food and their effects on drug metabolizing enzymes. Food Chem. Toxicol. 49, 3142–3153. doi:10.1016/j.fct.2011.09.015

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36. doi:10.1016/S0003-2670(00)88444-5

Norman, H.C., Masters, D.G., Barrett-Lennard, E.G., 2013. Halophytes as forages in saline landscapes: Interactions between plant genotype and environment change their feeding value to ruminants. Environ. Exp. Bot. 92, 96–109. doi:10.1016/j.envexpbot.2012.07.003

Oakes, K.D., Van Der Kraak, G.J., 2003. Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. Aquat. Toxicol. 63, 447–463. doi:10.1016/S0166-445X(02)00204-7

Okpala, C.O.R., Choo, W.S., Dykes, G.A., 2014. Quality and shelf life assessment of pacific white shrimp (*Litopenaeus vannamei*) freshly harvested and stored on ice. LWT - Food Sci. Technol. 55, 110–116. doi:10.1016/j.lwt.2013.07.020

Oliveira, G. da S., 2017. Avaliação de dois sistemas aquapônicos com a halófita *Sarcocornia ambigua* (Michx. Alonso & Crespo para recirculação de água da piscicultura marinha intensiva. Univerisdade Federal do Rio Grande.

Ouraji, H., Shabanpour, B., Kenari, A.A., Shabani, A., Nezami, S., Sudagar, M., Faghani, S., 2009. Total lipid, fatty acid composition and lipid oxidation of Indian white shrimp (*Fenneropenaeus indicus*) fed diets containing different lipid sources. J. Sci. Food Agric. 89, 993–997. doi:10.1002/jsfa.3545

Panta, S., Flowers, T., Lane, P., Doyle, R., Haros, G., Shabala, S., 2014. Halophyte agriculture: Success stories. Environ. Exp. Bot. 107, 71–83. doi:10.1016/j.envexpbot.2014.05.006

Patel, S., 2016. Salicornia: Evaluating the halophytic extremophile as a food and a pharmaceutical candidate. 3 Biotech 6, 1–10. doi:10.1007/s13205-016-0418-6

Piirainen, M., Liebisch, O., Kadereit, G., 2017. Phylogeny, biogeography, systematics and taxonomy of salicornioideae (amaranthaceae/chenopodiaceae) – a

cosmopolitan, highly specialized hygrohalophyte lineage dating back to the oligocene. Taxon 66, 109–132. doi:10.12705/661.6

Quideau, S., Deffieux, D., Douat-Casassus, C., Pouységu, L., 2011. Plant polyphenols: Chemical properties, biological activities, and synthesis. Angew. Chemie - Int. Ed. 50, 586–621. doi:10.1002/anie.201000044

Ríos-Durán, M.G., Valencia, I.R., Ross, L.G., Martínez-Palacios, C. a., 2013. Nutritional evaluation of autoclaved *Salicornia bigelovii* Torr. seed meal supplemented with varying levels of cholesterol on growth, nutrient utilization and survival of the Nile tilapia (Oreochromis niloticus). Aquac. Int. 21, 1355–1371. doi:10.1007/s10499-013-9638-5

Swingle, R.S., Glenn, E.P., Squires, V., 1996. Growth performance of lambs fed mixed diets containing halophyte ingredients. Anim. Feed Sci. Technol. 63, 137–148. doi:10.1016/S0377-8401(96)01018-8

Tomazelli Junior, O., Kuhn, F., Mendonça Padilha, P.J., Mota Vicente, L.R., Winckler da Costa, S., Corrêa da Silva, B., Dias Schleder, D., Boligon, A.A., Scapinello, J., Nunes Nesi, C., Dal Magro, J., De Lamo Castellví, S., 2017. Effect of *Cynodon dactylon* extract on white spot virus-infected *Litopenaeus vannamei*. Aquac. Int. 25, 1107–1122. doi:10.1007/s10499-016-0101-2

UNESCO, 1983. Chemical methods for use in marine environmental monitoring (No. 12), Intergovernmental Oceanographic Commission. Paris, France.

Ventura, Y., Eshel, A., Pasternak, D., Sagi, M., 2015. The development of halophyte-based agriculture: Past and present. Ann. Bot. 115, 529–540. doi:10.1093/aob/mcu173

Ventura, Y., Wuddineh, W.A., Myrzabayeva, M., Alikulov, Z., Khozin-Goldberg, I., Shpigel, M., Samocha, T.M., Sagi, M., 2011. Effect of seawater concentration on the productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia* halophytes as leafy vegetable crops. Sci. Hortic. (Amsterdam). 128, 189–196. doi:10.1016/j.scienta.2011.02.001

Xia, X., Kong, B., Liu, Q., Liu, J., 2009. Physicochemical change and protein oxidation in porcine longissimus dorsi as influenced by different freeze-thaw cycles. Meat Sci. 83, 239–245. doi:10.1016/j.meatsci.2009.05.003

Tabela 1. Composição proximal, compostos fenólicos e flavonoides totais na farinha de *Salicornia neei* cultivada em hidroponia com efluente da aquicultura.

Composição	Valor médio
Matéria seca (g $100g^{-1}$)	96,4
Matéria mineral (g $100g^{-1}$)	34,2
Proteína Bruta (g $100g^{-1}$)	7,9
Extrato etéreo (g $100g^{-1}$)	3,7
Fibra bruta (g $100g^{-1}$)	5,3
Extrativo não nitrogenado (ENN)[1]	48,9
Energia bruta (kJ $100g^{-1}$)[2]	1090
Fenóis totais livres (g EAG $100g^{-1}$)	2,75
Flavonoides totais livres (g EQ $100g^{-1}$)	1,01

[1]ENN = 100 – (proteína bruta + fibra bruta + extrato etéreo + cinza). [2]Energia bruta foi calculada considerando que cada grama de proteína bruta, extrato etéreo e ENN geram 16,7, 37,7 e 16,7 kJ, respectivamente. EAG - Equivlente de ácido gálico; EQ - Equivalente de quercetina.

Tabela 2. Ingredientes, composição proximal e compostos fenólicos determinados nas três dietas experimentais para *Litopenaeus vannamei* com diferentes níveis de inserção de *Salicornia neei*.

Ingredientes (g $100g^{-1}$)	0,0	15,0	30,0
Farinha de peixe[1]	45,1	45,1	45,1
Farelo de soja	5,0	5,0	5,0
Levedura de cerveja	5,0	5,0	5,0
Mistura mineral e vitamínica[2]	1,0	1,0	1,0
Colesterol[3]	0,5	0,5	0,5
$Ca(H_2PO_4)_2$[3]	2,0	2,0	2,0
Amido de milho	18,0	13,8	7,8
Farelo de trigo	11,5	6,5	1,4
Óleo de peixe[4]	3,2	2,7	2,2
Celulose[3]	8,7	3,4	0,0
Farelo de *Salicornia neei*	0,0	15,0	30,0
Composição			
Proteína bruta (g $100g^{-1}$)	34,9	33,5	34,3
Fibra bruta (g $100g^{-1}$)	7,2	5,6	5,5
Extrato etéreo (g $100g^{-1}$)	7,9	7,5	7,2
Matéria mineral (g $100g^{-1}$)	16,5	21,7	25,8
Extrativo não nitrogenado (ENN)[5]	33,4	31,6	27,2
Energia bruta (kJ $100g^{-1}$)[6]	1439	1371	1297
Fenóis totais livres (g EAG $100g^{-1}$)	0,036	0,047	0,055
Flavonoides totais livres (g EQ $100g^{-1}$)	nd	0,007	0,012

[1]Preparada pela mistura de farinha de peixe comercial (Torquato Pontes Pescado SA, Rio Grande, RS-Brasil) e farinha de restos de postas de Marlin *(Tetrapturus albidus*) adquiridos na feira livre da cidade de Rio Grande, RS-Brasil, na proporção de 1:4, respectivamente; [2]Premix M. Cassab, São Paulo, Brasil; [3]Vetec, Rio de Janeiro, Brasil; [4]Campestre, São Paulo, Brasil; [5]ENN = 100 – (proteína bruta + fibra bruta + extrato etéreo + cinza); [6]Energia bruta foi calculada considerando que a proteína bruta, extrato etéreo e ENN geram 16,7, 37,7 e 16,7 kJ g^{-1}, respectivamente*;* nd - Valor não detectado; EAG – Equivlente de ácido gálico; EQ -Equivalente de quercetina.

Tabela 3. Parâmetros zootécnicos dos camarões *Litopenaeus vannamei* alimentados com dietas com diferentes níveis de substituição de fontes tradicionais de carboidratos por farinha de *Salicornia neei*.

Dieta	PF	GP	GPS	FCA	TCE	TEP	Sobrevivência
0	9,53 ± 0,28	8,24 ± 0,28	1,37 ± 0,05	1,22 ± 0,03	4,44 ± 0,07	2,37 ± 0,08	100 ± 0,0
15	9,44 ± 0,27	8,15 ± 0,27	1,36 ± 0,04	1,23 ± 0,02	4,42 ± 0,06	2,44 ± 0,08	100 ± 0,0
30	8,96 ± 0,63	7,67 ± 0,63	1,28 ± 0,11	1,31 ± 0,10	4,30 ± 0,16	2,24 ± 0,19	100 ± 0,0

PF – Peso final em gramas; GP - Ganho de peso em gramas; GPS - Ganho de peso semanal em gramas semana^{-1}; FCA - Fator de conversão alimentar; TCE - Taxa de crescimento específico em % dia^{-1}; e TEP - Taxa de eficiência proteica. Não foram detectadas diferenças significativas nos parâmetros zootécnicos entre as dietas testadas (ANOVA; $p > 0,05$).

Tabela 4. Parâmetros de cromaticidade, L*a*b*, dos camarões *Litopenaeus vannamei* alimentados com dietas de diferentes níveis de inserção (0, 15 e 30%) de farinha de *Salicornia neei* após 1, 3, 6, 8 e 10 dias do abate (vida útil).

Parâmetro	Inserção (%)	1	3	6	8	10
*L**	0	$33,5 \pm 2,33^{Aa}$	$38,1 \pm 1,45^{Aa}$	$37,9 \pm 2,09^{Aa}$	$37,7 \pm 3,07^{Aa}$	$35,0 \pm 2,73^{Aa}$
	15	$33,4 \pm 0,28^{Aab}$	$37,7 \pm 0,17^{Aab}$	$32,2 \pm 0,80^{Aab}$	$39,0 \pm 4,41^{Aa}$	$30,3 \pm 2,62^{ABb}$
	30	$32,2 \pm 1,16^{Aab}$	$35,2 \pm 1,68^{Aa}$	$33,1 \pm 1,11^{Aab}$	$35,0 \pm 2,01^{Aa}$	$27,9 \pm 0,95^{Bb}$
a*	0	$-2,22 \pm 0,14^{Bb}$	$-2,40 \pm 0,23^{Bb}$	$-1,68 \pm 0,15^{Ba}$	$-3,56 \pm 0,13^{Cc}$	$-2,59 \pm 0,00^{Bb}$
	15	$-2,51 \pm 0,25^{Bb}$	$-1,75 \pm 0,06^{Aa}$	$-1,57 \pm 0,15^{Ba}$	$-2,63 \pm 0,14^{Bb}$	$-1,51 \pm 0,08^{Aa}$
	30	$-1,21 \pm 0,10^{Aab}$	$-1,49 \pm 0,15^{Ab}$	$-0,93 \pm 0,09^{Aa}$	$-1,14 \pm 0,06^{Aab}$	$-1,27 \pm 0,02^{Aab}$
b*	0	$-4,71 \pm 0,15^{Bc}$	$-5,08 \pm 1,71^{ABc}$	$-3,77 \pm 0,36^{Bbc}$	$0,70 \pm 0,43^{Aa}$	$-2,46 \pm 0,25^{Bb}$
	15	$-5,64 \pm 0,23^{Bbc}$	$-6,18 \pm 0,39^{Bc}$	$-4,20 \pm 0,30^{Bb}$	$-4,29 \pm 0,02^{Cb}$	$-2,43 \pm 0,12^{Ba}$
	30	$-2,57 \pm 0,12^{Ab}$	$-3,37 \pm 0,06^{Ac}$	$-2,35 \pm 0,26^{Abc}$	$-1,45 \pm 0,41^{Bb}$	$0,45 \pm 0,02^{Aa}$

L luminosidade (branco - valores positivos; preto – valores negativos), a* cromaticidade no espectro do vermelho (+) e verde (-), b* cromaticidade no espectro amarelo (+) e azul (-), respectivamente. Letras sobrescritas maiúsculas indicam diferenças estatísticas no teste de Tukey ($p<0,05$) entre os tratamentos (dietas). Letras sobrescritas minúsculas indicam diferenças estatísticas no teste de Tukey ($p<0,05$) entre os dias para o mesmo tratamento (dieta).

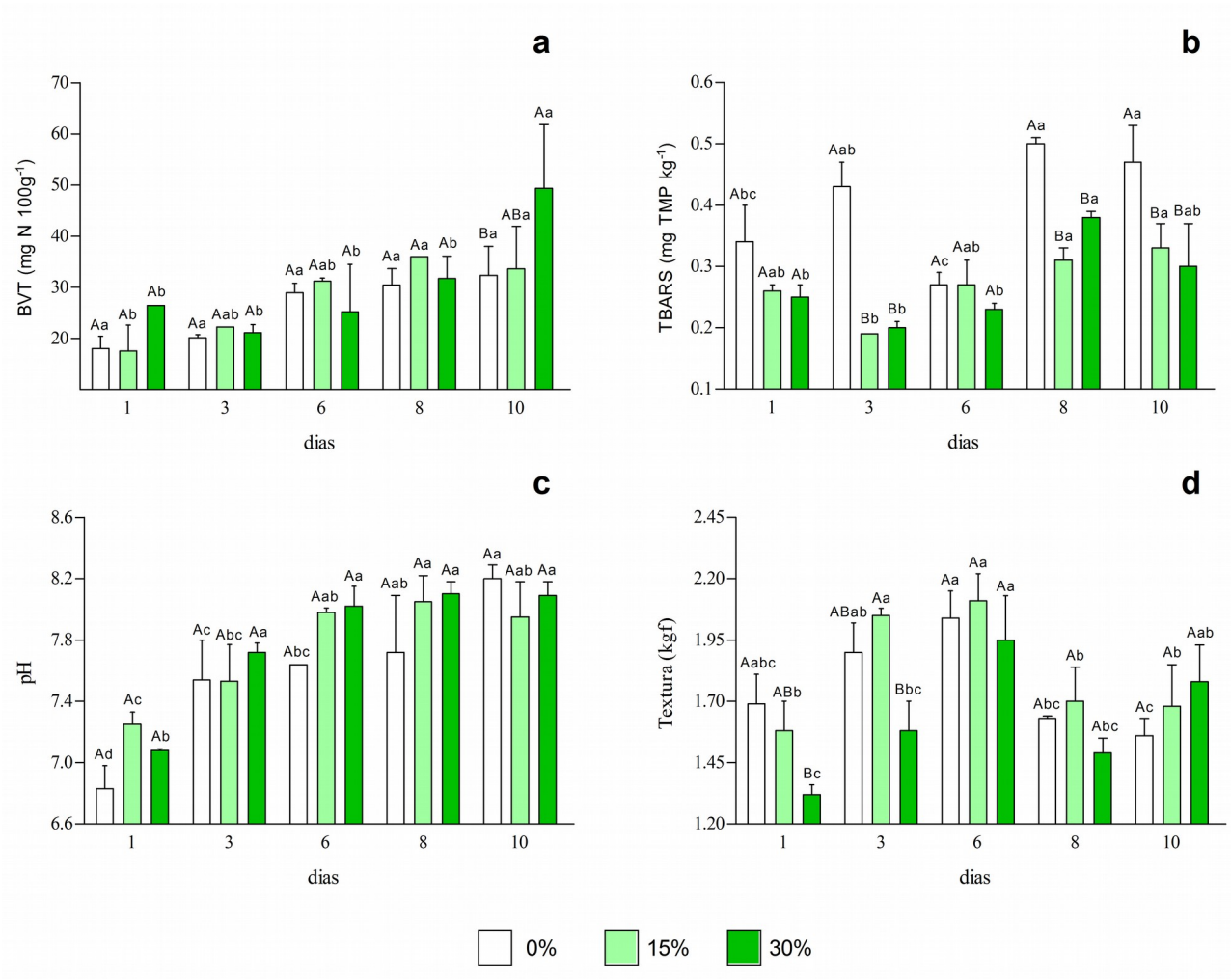


Figura 1. Os resultados de (a) bases voláteis totais (BVT), (b) peroxidação lipídica (TBARS), (c) pH e (d) textura na carne do *Litopenaeus vannamei* após 1, 3, 6, 8 e 10 dias de abatido com as dietas com 0, 15 e 30% de farinha de *Salicórnia neei*, genótipo BTH2. Letras sobrescritas maiúsculas indicam diferença estatística (teste de Tukey) entre as dietas no mesmo dia. Letras sobrescritas minúsculas indicam diferenças estatísticas (teste de Tukey) ao longo dos dias para cada dieta.

Discussão geral

Apesar dos elevados índices de produção, a aquicultura ainda possui espaço para crescimento em todo o mundo. Com destaque para a carcinicultura, que apesar de representar menos de 10% da produção aquícola mundial em peso, movimentou mais de 36 bilhões de dólares no ano de 2014 (quase 25% do valor monetário total para o mesmo ano). Contudo, o desenvolvimento da carcinicultura quando realizada de maneira inapropriada pode gerar diversos problemas socioeconômicos e ambientais, como o aporte de elevada carga de nutrientes orgânicos e inorgânicos para o ambiente. Esta problemática pode ser resolvida através da fitorremediação dos efluentes do cultivo com plantas halófitas. Além de assimilar os nutrientes da aquicultura, a biomassa vegetal produzida poderia ser um subproduto da atividade com alto valor agregado, uma vez que as halófitas apresentam boas características nutricionais e conteúdo de compostos funcionais.

Para acessar compostos funcionais, como os compostos fenólicos, em matrizes complexas como as halófitas (alto conteúdo de lignina, celulose e metais) faz-se necessário otimizar métodos químicos específicos para o grupo, como foi demostrado no Capítulo 1 deste trabalho. Além disso, o efeito de singular fonte de compostos bioativos também é verificado na sua capacidade antioxidante (Capítulo 2). Contudo, a extração destes compostos e sua utilização devem ser realizadas com parcimônia devido à complexidade da matriz vegetal halófita devido, possivelmente, ao elevado conteúdo de matéria mineral.

Com esta base de conhecimento na obtenção e utilização de compostos fenólicos nas halófitas, pudemos avançar os estudos no sentido de verificar o efeito das condições ambientais no crescimento e características nutricionais destas plantas, visto que o mercado consumidor espera um produto (seja ele alimento ou matéria prima para a indústria) com características constantes. O aspargo marinho *Salicornia neei* foi escolhido para testes de composição e produção devido ao Laboratório de Biotecnologia de Halófitas já ter amplo conhecimento fisiológico e de cultivo da espécie, desenvolvendo os novos genótipos BTH1 e BTH2. No Capítulo 3 foi confirmado que os novos genótipos de *S. neei* são halófitas constitutivas, que precisam de certo teor de sal

para obter melhor desempenho, com diferentes adaptações aos estresses oxidativo no fotossistema. Em cultivo de campo com efluente da carcinicultura, o genótipo BTH2 apresentou maior crescimento e conteúdo de compostos fenólicos. Neste capítulo ficou claro que as condições ambientais de cultivo podem influenciar a composição da planta, assim como seu desempenho fitotécnico. Sendo necessário o desenvolvimento de um protocolo de cultivo para que esta halófita mantenha suas características e possa aumentar seu consumo pelo mercado. Além disso, verificou-se que é possível obter altas produtividades de um alimento rico em compostos fenólicos a partir do efluente salino da carcinicultura.

Devido as características singulares das halófitas, seu fornecimento para animais poderia alterar o desempenho zootécnico e a vida útil da carne gerada? Esta pergunta foi respondida no Capítulo 4, onde a farinha do genótipo BTH2 da *S. neei,* produzida com efluente de aquicultura, foi inserida na ração dos camarões branco do pacífico *L. vannamei*. Apesar do elevado conteúdo de compostos fenólicos (positivo para o animal) e elementos minerais (que poderiam ter efeito negativo para o animal, pois por vezes são compostos antinutricionais) no genótipo BTH2 do aspargo marinho, não foi verificado efeito significativo no desempenho zootécnico e na sobrevivência do *L. vannamei*. A inserção da *S. neei* diminuiu os níveis de peroxidação lipídica e não afetou a vida útil da carne dos camarões cultivados. Contudo, trabalhos futuros avaliando a qualidade da carcaça e a sanidade do animal de forma direta (e.g. estresse oxidativo) são essenciais para uma compreensão mais profunda dos efeitos da farinha do genótipo BTH2 da *S. neei* na nutrição do *L. vannamei*. Os resultados do Capítulo 4 nos mostram que é possível utilizar farinha de *S. neei* como fonte de carboidrato e ácidos graxos, substituindo fontes tradicionais como o farelo de trigo e o óleo de peixe, respectivamente. O que pode resultar em diminuição de custo da produção, uma vez que a alimentação corresponde a mais de 50% dos gastos de cultivos aquícolas.

Conclusões gerais

Neste trabalho foi verificado que as halófitas brasileiras *Apium graveolens*, *Myrsine parvifolia*, *Paspalum vaginatum*, *Salicornia neei*, *Schinus terebinthifolius* e *Vigna luteola* apresentam elevado conteúdo de inúmeros grupos de compostos fenólicos (ácidos fenólicos, fenóis totais, flavonoides e quercetina) com elevada atividade antioxidante. Contudo, a extração destes compostos e sua utilização devem ser realizadas com parcimônia devido à complexidade da matriz devido, possivelmente, ao elevado conteúdo de elementos minerais.

Os genótipos de *S. neei* desenvolvidos no Laboratório de Biotecnologia de Halófitas apresentaram elevada tolerância ao estresse salino com diferentes adaptações no sistema fotossintético para dissipar o excesso de energia. Com o genótipo BTH2 apresentando melhor desempenho fitotécnico em cultivo de campo com efluente salinos da carcinicultura. Além disso, mostrou maior produtividade com efluente salinos do que cultivares tradicionais (aspargo comum, brócolis, espinafre a alcachofra) cultivados com água doce e em áreas agriculturáveis do globo, cada vez mais escassas.

O genótipo BTH2 não apresentou efeito negativo quando inserido em até 30% na ração do camarão branco do pacífico *Litopenaeus vannamei*. A inserção da *S. neei* diminuiu os níveis de peroxidação lipídica e não afetou a vida útil da carne dos camarões. Contudo, trabalhos futuros avaliando a qualidade da carcaça e a saúde do animal de forma direta (e.g. estresse oxidativo) são essenciais para um aprofundamento da compreensão dos efeitos da farinha do genótipo BTH2 da *S. neei* na nutrição do *L. vannamei.*

Em resumo, com o cultivo de plantas halófitas brasileiras, com destaque para a *Salicornia neei,* é possível produzir alimento e matéria-prima para a indústria química e farmacêutica utilizando água/efluente salino. Esta nova alternativa tecnológica pode acarretar em menores custos de produtos, pois o cultivo não ocorre em áreas agriculturáveis (que vem diminuindo nas últimas décadas), e menor degradação ambiental, uma vez que a produção de biomassa pode ser acoplada ao tratamento de efluentes.