UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INTITUTO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

Marina Rocha Komeroski

Qualidade nutricional, bioativa, microbiológica e sensorial de microgreens de rúcula (*Eruca sativa*) frescos e submetidos à diferentes embalagens e dias de armazenamento

Porto Alegre, 2024

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INTITUTO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

Marina Rocha Komeroski

Qualidade nutricional, bioativa, microbiológica e sensorial de microgreens de rúcula (*Eruca sativa*) frescos e submetidos à diferentes embalagens e dias de armazenamento

Projeto apresentado ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos da Universidade Federal do Rio Grande do Sul (PPGCTA/UFRGS) para obtenção do grau de doutora.

Orientadores: Profº Dr. Alessandro de Oliveira Rios e Profª Dra. Simone Hickmann Flôres

Co-orientadora: Profª Dra. Tâmmila Venzke Klug

Porto Alegre, 2024

CIP - Catalogação na Publicação

Komeroski, Marina Rocha Komeroski
Qualidade nutricional, bioativa, microbiológica e sensorial de microgreens de rúcula (Eruca sativa) frescos e submetidos à diferentes embalagens e dias de armazenamento / Marina Rocha Komeroski Komeroski. -- 2024.
124 f.
Orientador: Alessandro de Oliveira Rios Rios.

Coorientador: Tâmmila Venzke Klug Klug.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Instituto de Ciência e Tecnologia de Alimentos, Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, Porto Alegre, BR-RS, 2024.

1. microgreens. 2. compostos bioativos. 3. vida útil. I. Rios, Alessandro de Oliveira Rios, orient. II. Klug, Tâmmila Venzke Klug, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

DEDICATÓRIA

Aos meus pais, Leandro e Valéria, que sempre me estenderam a mão quando eu precisei. Se hoje eu finalizo minha trajetória como aluna da UFRGS, depois de 11 anos entre graduação, mestrado e doutorado, é porque recebi muito amor, apoio e incentivo de vocês. Obrigada por tudo!

AGRADECIMENTOS

Agradeço aos meus orientadores, Alessandro Rios e Simone Flôres, e à minha co-orientadora Tâmmila Klug, pelos ensinamentos em cada conversa e pela disponibilidade em me ajudar a resolver todos os percalços que apareceram no caminho. O meu muito obrigada à minha bolsista, Keyla Portal, que foi meu braço direito em muitas das análises realizadas, à minha amiga Raísa Homem, pela companhia e apoio nessa nossa jornada acadêmica, e ao meu grupo do Laboratório de Compostos Bioativos Laboratório 211 em especial à Cecília Roratto: vocês foram fundamentais nos momentos de desânimo e de dúvida. Obrigada pela ajuda e incentivo.

Deixo meus agradecimentos:

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelo auxílio financeiro concedido.

Ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos (PPGCTA) pelo suporte acadêmico.

A todos os professores e funcionários direta ou indiretamente envolvidos.

EPÍGRAFE

*“*Vulnerabilidade não é ganhar ou perder, é ter coragem
de agir quando não se pode controlar o resultado*”*

Brené Brown

RESUMO

Com uma produção sustentável, econômica e rápida, a popularidade dos microgreens está crescendo no âmbito comercial, e também nutricional, pelo seu alto teor de compostos bioativos, muitas vezes maior do que o presente no próprio vegetal em seu estágio maduro. No presente estudo foi realizado uma revisão da literatura sobre a influência dos sistemas de cultivo e o uso ou não de elicitores em microgreens da família Brassicaceae e também foi avaliado, comparativamente, o conteúdo de compostos bioativos e a composição centesimal de brotos e microgreens de rúcula (*Eruca sativa*) submetidos às mesmas condições de cultivo. Como resultado, esses vegetais apresentaram alto teor de proteína, sendo que os brotos exibiram valores superiores aos microgreens (24,8% brotos; 18,2% microgreens) e alto teor de fibras totais (34,3% brotos; 28,7% microgreens) e fibras solúveis (11,5% brotos; 6,7% microgreens). Em relação aos compostos bioativos, notou-se a predominância dos carotenoides zeaxantina e β-caroteno nos brotos (41,8 e 19,8 µg/g, respectivamente) e a identificação de outros compostos como O-glicosídeos derivados de flavonoides nos microgreens, tais como apigenina, isohamnetina, miricetina e quercitina. Em um segundo momento, os microgreens de rúcula foram armazenados a 5°C em embalagens aberta, selada à vácuo e sob atmosfera modificada por 0, 3, 5, 7 e 10 dias para analisar alterações de clorofila, coloração, pH, acidez, sólidos solúveis, perda de peso, conteúdo microbiológico e atributos sensoriais. Verificou-se que os microgreens armazenados em todas as embalagens mostraram-se microbiologicamente seguros para consumo por pelo menos 10 dias. A embalagem aberta e a sob atmosfera modificada mantiveram a qualidade nutricional durante o armazenamento. Essas amostras apresentaram atributos semelhantes às frescas, pois obtiveram aceitação similares na análise sensorial.

Palavras-chave: microgreens; brotos; vida útil, compostos bioativos.

ABSTRACT

With sustainable, economical and fast production, microgreens are becoming increasingly popular in the commercial and nutritional fields, due to their high content of bioactive compounds, often higher than that present in the vegetable itself at its mature stage. In this study, a literature review was carried out on the influence of cultivation systems and the use or not of elicitors in microgreens of the Brassicaceae family. The content of bioactive compounds and the centesimal composition of arugula (*Eruca sativa*) sprouts and microgreens subjected to the same cultivation conditions were also comparatively evaluated. As a result, these vegetables presented a high protein content, with sprouts exhibiting higher values than microgreens (24.8% sprouts; 18.2% microgreens) and a high content of total fiber (34.3% sprouts; 28.7% microgreens) and soluble fiber (11.5% sprouts; 6.7% microgreens). Regarding bioactive compounds, the predominance of carotenoids zeaxanthin and β-carotene in sprouts (41.8 and 19.8 µg/g, respectively) was observed, and other compounds such as O-glycosides derived from flavonoids, such as apigenin, isohamnetin, myricetin and quercitin, were identified in microgreens. In a second step, the arugula microgreens were stored at 5°C in open, vacuum-sealed, and under a modified atmosphere packages for 0, 3, 5, 7 and 10 days to analyze changes in chlorophyll, color, pH, acidity, soluble solids, weight loss, microbiological content and sensory attributes. It was found that the microgreens stored in all packages were microbiologically safe for consumption for at least 10 days. The open and modified atmosphere packages maintained their nutritional quality during storage. These samples presented attributes similar to the fresh ones, as they obtained similar acceptance in the sensory analysis.

Keywords: microgreens; sprouts; shelf life, bioactive compounds.

LISTA DE FIGURAS

ARTIGO 1

ARTIGO 2

ARTIGO 3

LISTA DE TABELAS

ARTIGO 1

ARTIGO 2

ARTIGO 3

LISTA DE ABREVIATURAS E SIGLAS

ABTS	2,2-azino-bis 2,2’-azino-bis- (ácido 3-etilbenzotiazolina-6-sulfonico)
ACP	Análise de Componentes Principais
AESA	Autoridade Europeia para a Segurança Alimentar
AOAC	*Association of Official Agricultural Chemists*
CAPES	Coordenação De Aperfeiçoamento De Pessoal De Nível Superior
CFU	Unidade Formadora de Colônias
CLAE	Cromatografia Líquida De Alta Eficiência
cm	Centímetro
DNA	Ácido Desoxirribonucleico
FDA	*Food and Drug Administration*
g	Grama
h	Hora
HCl	Ácido Clorídrico
HPLC	*High Performance Liquid Chromatography*
Kcal	Quilocalorias
KNO_3	Nitrato de Potássio
KOH	Hidróxido de Potássio
LED	*Light Emitting Diode*
log	Logaritmo
mg	Miligrama
mL	Mililitro
µL	Microlitro
µg	Micrograma
µMol/gdw	MicroMol por grama de peso seco
MTBE	Éter Terc-Metil-Butílico
NaOCl	Hipoclorito de Sódio
NaOH	Hidróxido de Sódio
Nm	Nanômetro
PPO	Polifenoloxidase
p/v	Partes por Volume
UR	Umidade Relativa
UV	Ultravioleta Visível
UV-B	Ultravioleta B

SUMÁRIO

CAPÍTULO 1

1. INTRODUÇÃO

Produzidos a partir de sementes de vegetais, ervas ou grãos, o que inclui espécies selvagens ou mesmo ornamentais, os microgreens são mudas colhidas quando as folhas cotiledonares estão totalmente desenvolvidas, com ou sem o surgimento de um par de folhas verdadeiras (Xiao et al., 2012). Geralmente são servidos frescos como ingrediente em saladas, sopas e sanduíches ou usados como guarnição comestível (Treadwell et al., 2010). Devido às suas múltiplas cores, texturas e sabores, esse novo produto alimentício tem aumentado sua popularidade frente aos consumidores (Mir et al., 2016).

Os microgreens começaram a ser comercializados na década de 80, nos Estados Unidos (Choe et al., 2018). Seu cultivo domiciliar também é recente, por isso, muitas vezes, são confundidos com brotos. No entanto, diferem em relação ao ciclo de crescimento, meio de cultivo, parte comestível, presença ou ausência de luz e de fertilizantes durante a produção (Di Gioia et al., 2017).

Quase todas as sementes vegetais podem ser usadas para o a produção de microgreens, mas a maioria das espécies comerciais desses vegetais são da família Brassicaceae, que são as mais consumidas em todo o mundo. Exemplos dessas espécies incluem brócolis, couve-flor, couve de Bruxelas, repolho, rabanete, nabo e couve-rábano (Xiao et al., 2019).

Os microgreens ao serem expostos à luz solar ou artificial produzem metabólitos secundários em resposta ao estresse gerado por esses raios. Muitos destes metabólitos, conhecidos como compostos bioativos, entre eles: compostos fenólicos, flavonoides e carotenoides, são benéficos para a saúde humana (Gui et al., 2018). A contribuição dos microgreens para a saúde pode ser atribuída também à capacidade antioxidante, além de uma vasta gama de nutrientes presentes, tais como: vitaminas A, C e do complexo B, minerais e fibras (Liu, 2013). Alguns estudos demostraram que os microgreens podem ter níveis muito mais altos dessas vitaminas, minerais e outros fitonutrientes quando comparado com os vegetais em seu estado de colheita tradicionalmente comercial (Xiao et al., 2012; Pinto et al., 2015; Mir et al., 2016; Singh et al., 2023).

Existem evidências científicas que sugerem que o consumo regular de vegetais é associado ao menor risco de desenvolver doenças crônicas não transmissíveis, como as doenças cardiovasculares, diabetes e obesidade (Xiao et al., 2012; Cheng et al., 2015). Estudos demonstraram ainda que, ao invés da suplementação isolada desses nutrientes, o corpo humano aproveita de uma melhor forma as interações desses fitoquímicos em suas diferentes fontes de origem (Yusuf et al., 2000; Liu, 2013).

Como um novo setor da indústria de vegetais, os microgreens estão se tornando cada vez mais predominantes, com sua comercialização em mercados de agricultores locais, lojas de alimentos especializados e em seções de produtos de grandes lojas de varejo; no entanto, há informações limitadas sobre os nutrientes e a composição fitoquímica neste estágio inicial de maturidade. À medida que a demanda do consumidor e a conscientização sobre o seu potencial aumentam, são necessárias informações nutricionais precisas dos microgreens disponíveis no mercado (Xiao et al., 2019).

Para a disseminação do uso de microgreens como uma alternativa vegetal viável é necessário compreender o efeito das condições de cultivo no acúmulo de compostos bioativos, a fim de se aperfeiçoar sua técnica de produção (Bulgari et al., 2016). Ainda, um melhor entendimento das culturas de microgreens e características pós-colheita e de armazenamento são cruciais para prolongar sua vida útil e para a expansão desses produtos no mercado (Mir et al., 2016).

2. OBJETIVOS

2.1 Objetivo geral

Avaliar as características nutricionais, bioativas, físico-químicas, microbiológicas e sensoriais de microgreens de rúcula (*Eruca sativa*) frescos e sob diferentes embalagens e dias de armazenamento.

2.2 Objetivos específicos

- ➔ Realizar uma revisão da literatura sobre a influência dos sistemas de cultivo e o uso ou não de elicitores em microgreens da família Brassicaceae.
- ➔ Caracterizar os microgreens de rúcula (*Eruca sativa*) em relação a composição centesimal, compostos bioativos, vitamina C, vitamina A e capacidade antioxidante.
- ➔ Avaliar a qualidade microbiológica, físico-química e sensorial de microgreens de rúcula (*Eruca sativa*) submetidos à diferentes embalagens (aberta, selada à vácuo e sob atmosfera modificada) e armazenados sob refrigeração em até 10 dias.

CAPÍTULO 2

3. REFERENCIAL TEÓRICO

3.1 Valor nutricional dos microgreens

Microgreens são vegetais folhosos jovens, colhidos quando as primeiras folhas se expandem completamente e geralmente antes que as folhas verdadeiras surjam. Essa cultura tem um rápido ciclo de produção, de uma a três semanas, e pode ser produzida em estufas, no solo ou, mais comumente, em sistemas sem solo, utilizando meios de cultivo sólidos orgânicos ou inorgânicos ou hidroponia (D'Imperio et al., 2024), o que demonstra o potencial desses produtos para adaptar a produção de vegetais folhosos a uma menor escala (Teng et al., 2023).

Consumidos majoritariamente frescos, os microgreens contêm quantidades significativas de importantes compostos bioativos e minerais (Paradiso et al., 2018), muitas vezes sendo superior ao das plantas adultas da mesma espécie (Butkutė et al., 2018), pois recebem apenas tratamentos leves na etapa de pós-colheita, como corte, embalagem e armazenamento refrigerado (Di Gioia et al., 2017). Além disso, representam uma opção rica em nutrientes para consumidores com uma alimentação mais restrita, como os veganos e crudívoros (Kyriacou et al., 2016).

A composição nutricional dos microgreens depende das condições e do tipo de cultivo, bem como da espécie semeada. Mas, de modo geral, estes parecem ser excelentes fontes de nutrientes e componentes bioativos, tais como: vitaminas (principalmente K, C e E), carotenoides, polifenois e glucosinolatos (Choe et al., 2018; Fusari et al., 2020).

Os microgreens são também boas fontes de diversos minerais, podendo servir como uma estratégia de promoção da saúde para atender ao consumo de referência na dieta. A presença e abundância de elementos minerais essenciais é um critério importante para a avaliação da qualidade nutricional e influencia a saúde humana (Xiao et al., 2016; Bhaswant et al., 2023).

De acordo com o Departamento de Agricultura dos Estados Unidos (USDA, 2014), os microgreens começaram a aparecer nos cardápios dos chefes de cozinha nos

anos 80, na Califórnia. Inicialmente, não havia muitas espécies oferecidas, apenas rúcula, manjericão, beterraba, couve e coentro. Atualmente uma vasta gama de microgreens é produzida (Alloggia et al., 2023), principalmente das famílias Brassicaceae, Asteraceae, Amarillydaceae, Amaranthaceae e Cucurbitaceae (Xiao et al., 2015).

3.2 Microgreens versus Brotos

Comumente confundidos com brotos, os microgreens também podem ser facilmente cultivados em ambientes urbanos ou peri-urbanos, onde a terra é muitas vezes um fator limitante, por agricultores especializados em hortaliças ou pelos próprios consumidores A diferença entre brotos e microgreens é que o primeiro é geralmente cultivado no escuro, sem um meio de crescimento e sua porção comestível é constituída por todo o broto, incluindo as raízes (Tabela 1). Por sua vez, os microgreens requerem luz e um meio de crescimento e, em relação à parte comestível, excluem-se as raízes (Di Gioia et al., 2017).

Tabela 1. Principais diferenças entre brotos e microgreens, adaptado de Di Gioia et al. (2017).

Estágio da planta	Ciclo de crescimento (dias)	Meio de cultivo	Porção comestível	Presença de luz	Uso de fertilizantes
Brotos	4-10	Sistemas sem solo	Toda planta	Não precisa	Não
Microgreens	7-28	Sistemas sem solo, estufas ou solo	Caule e cotilédones	Sim	A nível comercial

Além disso, os brotos são nutricionalmente superiores às sementes originais, pois além de possuírem maiores níveis de nutrientes, apresentam menor quantidade de anti-nutrientes e uma maior digestibilidade de proteínas e de amido (Zhang et al., 2015). A disparidade quanto à germinação é ilustrada na Figura 1.

Diferentemente dos microgreens, os brotos possuem regulamentação publicada pela *Food and Drug Administration* (FDA), órgão responsável por garantir segurança

à saúde pública, relativa à sua produção para venda comercial, devido ao seu risco relativamente alto de contaminação em comparação com o cultivo de outros vegetais (Ebert et al., 2022).

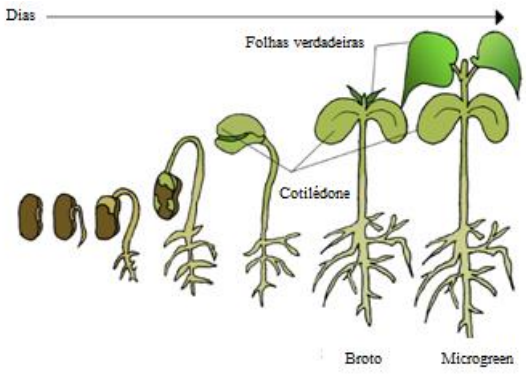


Figura 1. Período de germinação de brotos e microgreens, adaptado de Riggo et al., 2019.

3.3 Sistemas de cultivo

Os microgreens são geralmente produzidos em sistemas de cultivo sem solo, nos quais este é substituído por um substrato e as mudas são alimentadas com uma solução nutritiva contendo todos os elementos essenciais para o seu crescimento (Renna et al., 2016; D'Imperio et al., 2024). Dado seu ciclo de crescimento ser curto, essas mudas podem ser cultivadas organicamente (Ebert et al., 2022). Como o tempo entre a semeadura e a colheita de microgreens difere entre as espécies (Kyriacou et al., 2018), os produtores devem selecionar culturas que tenham uma taxa de crescimento semelhante, de modo que a produção possa ser colhida de uma só vez (Ebert et al., 2022).

Segundo Di Gioia et al. (2015b), a produção comercial é normalmente realizada em ambiente controlado, dentro de estufas, com o uso de sistemas de cultivo que podem ser de três tipos: a) cultivo dos microgreens em bandejas de plástico. Essa maneira permite a comercialização do produto logo após o plantio, o que evita o corte antes do consumo; b) uma segunda possibilidade é cultivar os microgreens em calhas, que podem ser de plástico, alumínio, ferro galvanizado ou madeira, com materiais (perlita, fibra de coco, etc) como um forro a ser colocado no fundo, proporcionando um leito de semeadura

(Ebert et al., 2014); c) a terceira alternativa é a produção de microgreens em um “sistema flutuante”. Nesse caso, são utilizadas bandejas de poliestireno que flutuam sobre a solução nutritiva contida em uma bacia ou em uma bancada. À nível comercial, essa é a opção menos utilizada, pois tem um menor prazo de validade (Di Gioia et al., 2015b). Nesse sistema hidropônico, a higienização de sementes é importante para o cultivo dos microgreens, pois permite um aumento notável na taxa de germinação e uma diminuição no crescimento de fungos (Andrews et al., 2019).

No que se refere às condições de cultivo, devem ser considerados a densidade de semeadura, o uso de fertilizantes e a dosagem de luz recebida (Choe et al., 2018; Lerner, Strassburguer & Schafer, 2024). Em relação a esse último fator, a intensidade de luz recebida pelas jovens mudas pode acarretar em um maior acúmulo de pigmentos fotossintéticos e de carotenoides (Samuolienė et al., 2017; Drygas et al., 2024). A utilização da luz LED, por exemplo, pode promover o acúmulo de compostos fenólicos, vitaminas, glucosinolatos, clorofila, carotenoides e fornecer uma maior capacidade antioxidante para as espécies cultivadas sob essa condição (Zhang et al., 2020).

A escolha de um meio de cultura com características microbiológicas adequadas é de extrema importância para garantir um consumo seguro de microgreens, já que o meio escolhido pode representar uma fonte de contaminação (Di Gioia et al., 2016). De acordo com esses pesquisadores, materiais fibrosos reciclados descartados da indústria de fibras têxteis, como as de juta e sisal, podem ser usados como substratos alternativos renováveis para a produção de microgreens, o que garante alto rendimento e qualidade.

3.4 Colheita e armazenamento

Como brotos e microgreens são geralmente consumidos crus, não há perda ou degradação de micronutrientes sensíveis ao calor através do processamento de alimentos (Ebert et al., 2022). Esses produtos podem ser considerados minimamente processados uma vez que sofrem simples etapas após a colheita, como lavagem, secagem e embalagem (Castro-Ibáñez & Allende, 2017). São preferencialmente consumidos imediatamente após a lavagem, mas também podem ser mantidos dentro de recipientes de vidro, fechados, por até uma semana sob refrigeração (Ebert, 2022).

O estágio de senescência após a colheita de microgreens é mais rápido devido à interrupção repentina do crescimento da planta em um estágio muito inicial. Como as microgreens são colhidas na fase de cotilédones, este processo pode resultar na degradação do produto primário. As duas técnicas mais utilizadas para aumentar a extensão da vida útil pós-colheita são a temperatura de armazenamento e as condições atmosféricas de armazenamento. A embalagem adequada também reduz a contaminação microbiológica do produto (Mir et al., 2016; Teng et al., 2023).

Em relação à vida útil dos microgreens, sua estrutura delicada e imatura demonstra-se pouco resistente à temperatura ambiente. Logo, temperaturas mais baixas (entre 1 e 5 °C) podem prolongar a vida útil, bem como impactar positivamente a qualidade visual dessas mudas (Mir et al., 2016). Além disso, a maioria das culturas exige pouco ou nenhum fertilizante, pois a semente fornece a nutrição adequada para a planta, que será colhida poucos dias após o seu plantio (Xiao et al., 2015). Sendo assim, diante das variáveis de ambiente, sistema de cultivo e, consequentemente, escala de produção, seu prazo de validade é oscilante (Kyriacou et al., 2016).

Como não existem normas regulamentadoras específicas para microgreens, é necessário um equilíbrio para manter a temperatura, a umidade e a atmosfera, de modo que otimizem a retenção de qualidade e a vida útil dos microgreens, e preferencialmente dificultem o crescimento de microorganismos deteriorantes e patógenos humanos (Turner, 2020).

Para identificar a temperatura ideal de armazenamento, Dayarathna et al. (2023) avaliaram o efeito de diferentes temperaturas de armazenamento na qualidade de pós-colheita de microgreens de mostarda armazenados em embalagens seladas de polietileno. Quando armazenados a 5 °C, os microgreens de mostarda não apresentaram alterações significativas na atividade antioxidante e mantiveram boa qualidade sensorial geral por 14 dias. As amostras armazenadas a 10 e 15 °C mantiveram boa qualidade sensorial geral por quatro e dois dias, respectivamente. Já quando armazenados a 20 e 25 °C, os microgreens se deterioraram em um dia.

O efeito da embalagem de atmosfera modificada (MAP) associada à tratamentos químicos foi avaliado no trabalho de Patil et al. (2024). Microgreens de brócolis tratatos

com ácido ascórbico + ácido cítrico (0,25% p/v cada), juntamente com as condições da MAP (15% CO_2 + 5% O_2 + N_2 balanceado), retiveram significativamente ($p < 0,05$) fenóis totais (1247,68 mg GAE/100 g de peso fresco), flavonoides (56,9 mg QE/100 g peso fresco) e clorofila (42,12 mg/100 g de peso freso). Além disso, a utilização dessa embalagem suprimiu significativamente ($p < 0,05$) a perda de peso e reduziu a carga microbiana por até 12 dias.

Chandra et al. (2012) analisaram o desempenho pós-colheita a 5 °C de microgreens de repolho chinês 'Tah Tasai' (*Brassica campestris* var. narinosa) armazenados em embalagens de polietileno e polipropileno de maior e menor permeabilidade a gás, respectivamente, e verificaram que os filmes de polipropileno, devido ao maior acúmulo de CO_2, causaram danos à membrana mais rápidos e irreversíveis, inferidos pelo aumento do vazamento de eletrólitos e pelo odor desagradável.

De acordo com Mir et al. (2016), os avanços na tecnologia de embalagem irão contribuir com a manutenção da qualidade de microgreens por períodos mais longos e, consequentemente, estender sua vida útil. Além dos parâmetros de qualidade, as informações funcionais dessas plantas ajudarão a selecionar a cultura específica para determinado tipo de armazenamento.

No trabalho de Kou et al. (2013), a temperatura de armazenamento foi o fator mais importante para a manutenção da qualidade de microgreens de trigo sarraceno, seguido pelo tratamento de lavagem e, então pela embalagem sob atmosfera modificada, que teve efeito na integridade da membrana do tecido foliar. Segundo os autores, tanto as embalagens de baixa e de alta densidade de polietileno forneceram O_2 suficientemente alto e CO_2 suficientemente baixo para manter a qualidade aceitável dos microgreens.

Os efeitos combinados da luz ultravioleta (UV- C) e MAP com $O_2 > 85\%$ na rúcula fresca proporcionaram redução de cerca de 1 e 1,5 unidades logarítmicas nas contagens mesófilas e psicrotróficas, respectivamente, em 10 dias de armazenamento. Ainda, percebeu-se um aumento notável na capacidade antioxidante total e no teor de polifenóis, sem afetar a aparência visual das folhas (Silveira et al., 2015).

3.5 Rúcula

A família Brassicaceae, que compreende os vegetais crucíferos como a rúcula, é considerada a principal contribuinte para a produção e o consumo de vegetais no mundo. As crucíferas são reconhecidas como uma família rica em antioxidantes, vitaminas A e C e carotenoides (Alruwaih et al., 2017). As diferentes espécies de rúcula, *Eruca sativa*, *Diplotaxis tenuifolia e Diplotaxis muralis*, também contêm grandes concentrações de compostos flavonóis poliglicosilados, que são conhecidos por inferir numerosos benefícios para a saúde, principalmente ao trato gastrointestinal e ao sistema cardiovascular (Björkman et al., 2011).

Originalmente encontrada nos países do Mediterrâneo e do Oriente Médio, a rúcula ganhou popularidade na dieta ocidental devido ao seu aroma e sabor pungentes (Bell & Wagstaff, 2014), que pode ser explicado pela presença de glucosinolatos, isotiocianatos e seus derivados (glucosativina) (Passini et al., 2012). A rúcula é mais consumida crua, em saladas, mas também é servida em outros pratos, como pizzas. A cultivar brasileira *Eruca sativa* é irregular, de folhas largas, baixa estatura e anual periodicidade (Tassi et al., 2018).

Apesar dos inúmeros benefícios, quantidades significativas de rúcula são desperdiçadas anualmente devido à falta de processamento e às instalações de armazenamento (Ahmed et al., 2013). Ainda, esse vegetal é caracterizado por uma taxa de respiração extremamente alta e rápida senescência após a colheita, o que resulta em uma vida pós-colheita muito curta (Koukounaras et al., 2010), de aproximadamente 8 - 12 dias, quando armazenada em condições ideais (0°C e 100% de umidade relativa) (Tomás-Callejas et al., 2012).

Os microgreens de rúcula são normalmente consumidos em saladas, como guarnições ou adições a outros produtos para conferir sabor adicional e apelo estético. A concentração total de ácido ascórbico da rúcula é de aproximadamente 45,8 ± 3,0 mg 100 g^{-1} de peso fresco. Kathi et al. (2022) propuseram aumentar o nível de vitamina C a fim de fornecer uma opção mais acessível para a população. A biofortificação com ácido ascórbico na solução nutritiva resultou no consumo da recomendação diária dessa

vitamina com um volume muito menor de microgreens em comparação com a rúcula madura.

No trabalho de Bulgari et al. (2016), microgreens de rúcula obtiveram o maior valor para concentração de cálcio (18 $g.kg^{-1}$ de peso seco) e para clorofila a, b e total (0,74; 0,26 e 1,00 $mg.g^{-1}$ de peso fresco, respectivamente). Além disso, essas plantas apresentaram o maior conteúdo de açúcar quando comparadas com microgreens de acelga e de manjericão. Em particular, o conteúdo de sacarose na rúcula foi de 0.137 $mg.g^{-1}$ de peso fresco. Os açúcares são uma importante fonte de energia para manter o metabolismo celular. Após a colheita, eles são essenciais para manter as células vivas e garantir uma longa vida útil do produto.

A luz LED foi recomendada como um tratamento (por 6 h) pós-colheita para aumentar os compostos bioativos de microgreens de rúcula, repolho e rabanete no estudo de Ntsoane et al. (2024), aumentando significativamente as concentrações de ácido ascórbico, fenóis totais, kaempferol e glicosídeo de quercetina em todos os três microgreens de Brassica e atividades antioxidantes durante o armazenamento de cinco dias a 5 °C e com 85% de umidade relativa em comparação com as amostras recém-colhidas.

CAPÍTULO 3

4. MATERIAL E MÉTODOS

As sementes de rúcula (*Eruca sativa* L.) foram adquiridas da Isla Sementes Ltda. (Rio Grande do Sul, Brasil). O cultivo foi realizado no Laboratório de Compostos Bioativos do Instituto de Ciência e Tecnologia de Alimentos da Universidade Federal do Rio Grande do Sul (ICTA/UFRGS), conforme metodologia proposta por Di Gioia et al. (2017), com algumas modificações.

As sementes foram colocadas em espumas fenólicas com furos centrais e embebidas em água da torneira por 48 h para promover a germinação conforme recomendação do fornecedor. As placas de espuma fenólica semeadas foram colocadas sobre placas perfuradas de poliestireno para criar um sistema hidropônico flutuante e irrigadas com solução nutritiva preparada com água potável contendo: 6% de nitrogênio, 9% de fósforo, 29% de potássio, 2,7% de magnésio, 5% de enxofre, 0,2% ferro, 0,05% de manganês, 0,02% de zinco, 0,05% de boro, 0,03% de cobre, 0,002% de cobalto, 0,006% de níquel e 0,01% de molibdênio. Foi utilizada uma bomba de ar para oxigenar a solução nutritiva. As bandejas semeadas foram mantidas a 25°C em ambiente controlado e expostas à luz solar por fotoperíodo de 12 horas. Os brotos foram colhidos pela raiz após sete dias de germinação, enquanto as microgreens foram colhidas após 14 dias, cortando as plantas na base. O corte foi feito manualmente com uma tesoura em alguns milímetros acima da espuma fenólica.

O acondicionamento das amostras foi realizado em diferentes formas de embalagem de polipropileno (10 x 15 cm): embalagem aberta, o saco plástico permanece com o lado superior aberto; embalagem selada à vácuo, utilizando a seladora Fastvac® (modelo TM 250, TecMaq, São Paulo - Brasil) e embalagem com atmosfera modificada, no mesmo equipamento, com a seguinte composição de gases: 5% gás carbônico, 3% oxigênio e 92% nitrogênio, como preconizado por Chitarra & Chitarra (2005).

As embalagens continham em média 30 g de microgreens de rúcula e foram mantidas à temperatura de 5°C por até 10 dias em câmara incubadora BOD® (modelo NL-41-ALT, New Lab, São Paulo - Brasil) sob fotoperíodo de 12 h (ciclo claro/escuro).

4.1 Composição centesimal

A determinação da composição centesimal seguiu metodologia da AOAC (1995). Todas as análises foram realizadas em triplicata.

4.1.1 Umidade

A umidade foi avaliada a partir da perda de peso por dessecação de uma amostra de 1 g submetida ao aquecimento em estufa à 105 ºC (DeLeo®, Porto Alegre, Brasil) até a obtenção de peso constante.

4.1.2 Cinzas

As cinzas foram apuradas a partir do método gravimétrico de obtenção da perda de peso do material quando submetido à temperatura de 550 ºC (Linn High Therm®, Elektro, Eschenfelden, Alemanha). Uma amostra de 1 g foi pesada, seca em chapa de aquecimento e transferida para a mufla até a completa destruição da matéria orgânica, com a obtenção de peso constante. Em seguida, a amostra foi esfriada em dessecador e pesada em balança analítica.

4.1.3 Proteínas

A determinação de proteínas foi realizada pelo método Kjeldahl, utilizando 0,5 g de amostra, 5 g de mistura catalítica e 20mL de ácido sulfúrico para a etapa da digestão à 400 ºC em tubos de Kjeldahl. A partir de uma reação de oxirredução, as moléculas de proteína foram destruídas, liberando compostos voláteis, alterando a cor da amostra para uma tonalidade azulada. Na destilação, aproximadamente 60 mL de ácido bórico 4%, água destilada e indicador de Tashiro foram adicionados ao erlenmeyer. Em seguida, foi feita a titulação com ácido sulfúrico 0,1 N. O volume gasto foi anotado para medir o nitrogênio total e o valor foi convertido em proteína bruta pelo fator de referência 6,25.

4.1.4 Lipídeos

Os lipídeos foram determinados pelo método Bligh-Dyer. Por meio da mistura de três solventes (água, clorofórmio e metanol) em diferentes proporções foram formadas

duas fases distintas: uma de clorofórmio, onde ficam os lipídeos, e outra de metanol e água contendo compostos não lipídicos. A fase de clorofórmio foi então separada num balão para os lipídeos serem quantificados.

4.1.5 Fibras

A quantidade de fibras foi determinada de acordo com o método enzimático gravimétrico, na qual as amostras foram submetidas à digestão com três enzimas: alfa-amilase termo resistente, protease e amiloglicosidase, obtidas com o uso do Kit Sigma-Aldrich (modelo TDF100A-1KT).

Para determinação de fibras totais, após a digestão das amostras, foi adicionado álcool etílico 95%. Para determinação de fibras insolúveis seguiu-se o mesmo método, no entanto, sem a adição de álcool 95%. As fibras solúveis foram calculadas pela diferença.

4.1.6 Carboidratos

Os carboidratos foram avaliados pela diferença entre 100 g do alimento e a soma total dos valores a serem encontrados para proteínas, lipídeos, fibras e cinzas.

4.2 Coloração

As amostras de microgreens foram medidas por meio de um colorímetro da marca Konica Minolta® - Osaka/Japão (modelo Chrona Meter CR400). Este equipamento identifica o espectro de cores em um sistema tridimensional, sendo o eixo vertical, “L”, referente à cor da amostra do preto ao branco; eixo “a”, do verde ao vermelho; e eixo "b" de azul ao amarelo. O eixo L varia de 0 a 100, sendo que valores acima de 50 caracterizam as amostras mais claras e abaixo de 50, as mais escuras. Todas as leituras foram realizadas em triplicata.

4.3 pH, Sólidos solúveis e Acidez titulável

Os microgreens foram triturados em homogeneizador de tecidos Ultra Turrax® (modelo IKA T25) na proporção de 1:5 (folhas:água), filtrados e seu pH foi medido a 25

°C utilizando um medidor de pH Satra® (modelo pHS-3E) até obter um valor constante, conforme metodologia AOAC (1995). Já o °Brix é medido em refratômetro Atago® (modelo PAL-3). A acidez titulável foi medida titulando alíquotas de 10 mL de extratos de suco de microgreen com 0,1 mol/L de NaOH até o ponto final de pH 8,2, monitorado com um medidor de pH (medidor de pH Satra® (modelo pHS-3E)) em 21 ºC. Os resultados foram expressos como as concentrações de [H+] na unidade de mol/L com base na massa fresca da amostra (Xiao et al., 2015).

4.4 Perda de peso

A perda de peso foi realizada pesando os microgreens antes e depois do armazenamento. A perda de peso foi calculada de acordo com a seguinte fórmula (Yan et al., 2022):

$$\text{Perda de peso (\%)} = (m0 - m1)/m0 \times 100\%$$

Na qual:

m0 = o peso (g) das microgreens antes do armazenamento

m1 = o peso (g) de microgreens após armazenamento

4.5 Vitamina C

A determinação de vitamina C foi baseada na metodologia proposta pela AOAC (2006), com algumas modificações. Amostras de 1 g foram moídas em Ultra-Turrax com 20 mL a 0,05 M de ácido sulfúrico suprapuro a 96% (Merck), durante 1 min, centrifugadas a 25.400 g durante 15 min e, em seguida, filtradas através de uma unidade de filtro hidrofílico de teflon. Na sequência, as amostras foram analisadas por cromatografia líquida de alta eficiência (CLAE), com uma coluna polimérica C18 (250 mm x 4,6 mm i.d., 5 µm), e fase móvel de 0,05 M de ácido sulfúrico suprapuro a 1,0 mL/min, volume de injeção de 10 µL e comprimento de onda de 254 nm.

4.6 Vitamina A

A atividade da vitamina A foi calculada de acordo com NAS-IOM (2001), com base na atividade equivalente de retinol ($\mu g.RAE^{-1}$). Este sistema de conversão de carotenoides pró-vitamina A em vitamina A considera: 1 equivalente de atividade de retinol = 12 μg de β-caroteno; 1 equivalente de atividade de retinol = 24 μg de outros carotenoides pró-vitamina A (α-caroteno, β-criptoxantina e γ-caroteno). Assim, foram considerados neste estudo os seguintes carotenoides: β-caroteno, αcaroteno e β-criptoxantina.

4.7 Capacidade antioxidante

A determinação da capacidade antioxidante total dos brotos e microgreens foi determinada pela metodologia de captura do radical livre ABTS (2,2-azino-bis 2,2'-azino-bis- (ácido 3-etilbenzotiazolina-6-sulfonico), segundo Rufino et al. (2007). A leitura foi realizada a 734 nm em espectrofotômetro (Shimadzu® UV-1800), após 6 min da mistura.

4.8 Compostos bioativos

4.8.1 Carotenoides

O extrato de carotenoides foi preparado de acordo com Mercadante et al. (1998). As etapas principais foram: extração dos pigmentos com acetona e saponificação com metanol 10% KOH durante uma noite à temperatura ambiente.

As análises foram realizadas em um cromatógrafo Agilent®, série 1100 (Santa Clara, CA, EUA), equipado com um sistema solvente quaternário de bombeamento e um detector UV-Visível. A coluna usada para carotenoides foi de fase reversa C30 polimérica (YMC, modelo CT99SO3-2546WT). A fase móvel foi: água/metanol/éter metil-terc-butílico (MTBE) (JTBaker, Cas. Número 1634/04/04, pureza de 99,96%) a partir de 5:90:5, atingindo em 12 min 0:95:5, em 25 min, 0:89:11, 0:75:25, em 40 min e, finalmente, 00:50:50 depois de um total de 60 min, com uma taxa de fluxo de 1 mL/min a 33 ºC. Os espectros foram realizados entre 250 e 600 nm e os cromatogramas transformados em um comprimento de onda fixo de 450 nm para carotenoides.

A identificação foi efetuada por comparação dos tempos de retenção dos picos da amostra e do controle nas mesmas condições.

4.8.2 Compostos fenólicos

O conteúdo fenólico total foi determinado pelo método de Folin-Ciocalteu, seguindo Rodrigues et al. (2013). As amostras foram adicionadas ao meio solvente de extração na proporção de 1:120 (m/v). O solvente de extração compreendeu 0,35% v/v de solução de ácido fórmico em 20% v/v de acetona em água destilada. A pasta foi concentrada em evaporador rotativo para remoção da acetona. A extração foi repetida cinco vezes, mesmo sem cor do extrato. Uma alíquota de 0,5 mL do extrato da amostra foi misturada com 1,8 mL do reagente Folin-Ciocalteu 0,1 N (Sangon Biotech, Xangai, China). Após incubação durante 6 min à temperatura ambiente, a reação foi interrompida pela adição de 1,2 mL de uma solução aquosa de carbonato de sódio a 7,5%. Em seguida, a absorbância foi medida a 765 nm e conduzida em triplicata. O ácido gálico foi utilizado como padrão para uma curva de calibração e os resultados foram expressos como equivalentes de ácido gálico.

Para identificação e quantificação dos fenólicos, foi realizada extração exaustiva dos compostos fenólicos (Rodrigues et al., 2013). Para isso, foram adicionados 20 mL de uma mistura de metanol com água destilada (80:20; v/v) aos 0,4 g de amostra liofilizada, seguido de homogeneização em turrax por 2 mins, em temperatura ambiente (21 °C). Após o extrato ser centrifugado (Hitach CR21 GIII-Himac) a 10.000 g por 10 min a 20 °C, o sobrenadante foi transferido para um balão volumétrico âmbar. Este procedimento foi realizado em triplicata e repetido cinco vezes até a ausência de cor. A identificação dos compostos fenólicos foi feita em cromatografia HPLC Shimadzu (Kyoto, Japão), equipada com duas bombas (Shimadzu LC-20AD), desgaseificador (Shimadzu DGU-20A), forno de coluna (Shimadzu CTO-20A), sistema conectado a um detector de diodo de matriz (Shimadzu SPD-M20A) e um espectrômetro de massa (MS) com analisador quadrupolo – tempo de voo (Q-Tof) e fontes de ionização por eletrospray (ESI) (modelo micrOTOF-QIII, Bruker Daltonics, Bremen, Alemanha).

Foi utilizada coluna C18 Phenomenex SynergiTM (250 mm x 4,6 mm, 4 μm) (Allcrom, SP, Brasil). A fase móvel A foi composta por uma mistura de água Milli-Q ® e ácido fórmico 99,5:0,5% (v/v) e a fase móvel B uma mistura de acetonitrila e ácido fórmico 99,5:0,5% (v/v), em um gradiente linear, começando em 99:1 (v/v) fase móvel A/B, atingindo 50:50 (v/v) A/B em 50 mins, e então 50:50 (v/v) A/B para 1:99 (v/v) A/B por 5 min. Essa relação foi mantida por mais 5 min com vazão de 0,7 $mL.min^{-1}$ a 29 °C e o volume de injeção foi de 5 μL.

Os espectros foram obtidos entre 200 e 600 nm e os cromatogramas foram processados em 280, 320, 360 nm e 520 nm. Os espectros de massa foram adquiridos com varredura de m/z 100 a 1000. Os parâmetros MS foram definidos: fonte ESI nos modos de íons positivos (antocianinas) e negativos; tensão capilar 3000 V; temperatura do gás seco (N2) 310°C; vazão 8 L.min-1; gás nebulizador, 30 psi. MS2 foi configurado para modo automático. Os compostos fenólicos foram identificados com base na ordem de eluição e tempo de retenção na coluna, características dos espectros UV-VIS e MS comparados aos padrões analisados nas mesmas condições e dados disponíveis na literatura. Além disso, os compostos foram quantificados por HPLC-DAD-MS/MS utilizando uma curva analítica padrão.

4.8.3 Clorofila total, a e b

As amostras de rúcula (0,4 g) foram homogeinizadas no Turrax® (T25, IKA, China) junto com 5 mL de acetona, água (80:20) e então centrifugadas (SIGMA® 4K 15, USA) a 5000 g por 15 min. O sobrenadante foi utilizado para determinar o conteúdo de clorofila de acordo com as equações de Lichtenthaler & Wellburn. (1987). As leituras de absorbância no espectrofotômetro (Shimadzu® UV-1800, Japão) foram medidas em 663 nm para clorofila “a” e 647 nm para clorofila “b”, conforme Bulgari et al. (2016).

Na qual:

$CHL\ a\ (\mu g/ml) = 12.25 \times A_{663} - 2.79 \times A_{646}$

$CHL\ b\ (\mu g/ml) = 21.50 \times A_{646} - 5.10 \times A_{663}$

Total CHLs (μg/ml) = CHL a + CHL b

4.9 Análise sensorial

Microgreens frescos (0 dias) e armazenados (3, 5 e 7 dias) foram submetidos à análise sensorial no Laboratório de Avaliação Sensorial do Instituto de Ciência e Tecnologia de Alimentos (ICTA) da UFRGS em três dias, avaliando a aceitação dos seguintes atributos: aparência, textura, sabor, odor, cor, aceitação global e intenção de compra.

Foram recrutados aleatoriamente e voluntariamente 40 avaliadores adultos de ambos os sexos nas dependências da UFRGS para cada dia, convidados por cartazes fixados no ICTA e por folders virtuais nas redes sociais (Apêndice A).

Cada provador recebeu aproximadamente 1 g de cada amostra, com códigos aleatórios de três dígitos, um copo de água para a limpeza das papilas gustativas e uma ficha de avaliação para análise sensorial (Apêndice B). Os provadores foram questionados em relação à aceitação dos atributos seguindo uma escala hedônica de 9 pontos (1 = desgostei muito, 9 = gostei muito).

Este procedimento foi aprovado pelo comitê de ética e pesquisa da UFRGS e inserido na Plataforma Brasil. Número do Certificado de Apresentação de Revisão Ética: 65303222.5.0000.5347. Os provadores, para participarem da análise sensorial, assinaram o Termo de Consentimento Livre e Esclarecido (Apêndice C).

Para calcular o índice de aceitabilidade (IA) de cada tratamento foi utilizada a seguinte expressão, conforme descrita por Viana (2009):

$$IA\ (\%) = A \times 100 / B \quad (1)$$

Na qual:

A = nota média obtida em cada tratamento

B = nota máxima dada para cada tratamento

4.10 Análises microbiológicas

As análises microbiológicas foram realizadas em parceria com o Laboratório de Microbiologia do Instituto de Ciência e Tecnologia de Alimentos (ICTA/UFRGS).

4.10.1 Salmonella spp.

O isolamento de *Salmonella* spp. em amostras de microgreens armazenados em 5 ºC em câmera BOD® em embalagens abertas, seladas e com atmosfera modificada pelo período de 10 dias foi realizado segundo a metodologia ISO 6579-1:2017/Amd 1:2020. Para o pré-enriquecimento, 10 g da amostra foram adicionados em 90 mL de água peptonada tamponada (BPW; HIMEDIA) e foram incubados a 37 ºC por 24 h. Posteriormente, foi feito o enriquecimento seletivo em Caldo Tetrationato Muller Kauffmann (MKTTn; HIMEDIA) a 37 ºC por 24 h e Caldo Rappaport-Vassilidis Soja (RVS; HIMEDIA) a 37 ºC e 43 ºC por 24 h. Em seguida realizou-se a semeadura em placas de Ágar Xilose Lisina Desoxicolato (XLD; KASVI) e Hektoen Enteric Agar (HE; HIMEDIA), sendo ambos incubados a 37 ºC por 24 h (Silva et al., 2021).

Colônias típicas foram selecionadas e submetidas à identificação por meio da análise proteômica através de espectrometria de massa por dessorção/ionização por laser assistido por matriz e tempo de voo (MALDI-TOF/MS), modelo Autoflex Speed (Bruker Corporation, Bremen, Alemanha). As colônias selecionadas em XLD e HE foram transferidas para um tubo de microcentrífuga contendo 300 µL de água ultrapurificada mili-Q, sendo homogeneizadas em vórtex até ficarem turvas e, em seguida, foram adicionados 900 µL de etanol absoluto e homogeneizado. Os tubos foram encaminhados para o Instituto de Ciências Básicas da Saúde (ICBS/UFRGS) para análise (Barbosa et al., 2022).

4.10.2 Contagem total de Enterobacteriaceae e Escherichia coli

A contagem total de Enterobacteriaceae e *Escherichia coli* em amostras de microgreens armazenados em embalagens abertas, seladas e sob atmosfera modificada pelo período de 0 e 10 dias foi realizado segundo o método do Petrifilm™ (AOAC, 1995).

Para isso, 10 g da amostra foram homogeneizadas em 90 mL de tampão fosfato pH (7,2) (diluição 10^{-1}), em seguida foi feita a verificação do pH (6,5 a 7,5). A partir dessa primeira diluição foram preparadas diluições decimais seriadas da amostra (até 10^{-5}). Em seguida, foram selecionadas três diluições adequadas de cada amostra e de cada diluição foi inoculado 1 mL em uma placa de Petrifilm (PETRIFILM EC *E. coli* / Coliform). Posteriormente as placas foram incubadas a 35 ºC por 24 h para contagem de coliformes totais e 48 horas para contagem de *Escherichia coli* (Silva et al., 2021).

4.10.3 Listeria monocytogenes

O isolamento de *Listeria monocytogenes* em amostras de microgreens armazenados em embalagens abertas, seladas e com atmosfera modificada pelo período de 0, 5 e 10 dias foi realizado segundo a metodologia ISO 11290-1:2017. Para o pré-enriquecimento, 10 g da amostra foram adicionados em 90 mL de caldo Half-Fraser (HIMEDIA) e foram incubados a 30 ºC por 25 h. Posteriormente realizou-se a semeadura em placas de Ágar Listeria Ottaviani & Agosti (ALOA) e OXFORD, sendo ambos incubados a 37 ºC por 24 a 48 h. Além disso, 100 µL do conteúdo do tubo de pré-enriquecimento foram transferidos para 10 mL de caldo fraser e foram incubados a 37 ºC por 24 h. Após, foi realizado semeadura em placas ALOA e OXFORD incubadas a 37 ºC por 24 a 48 h (Silva et al., 2021).

4.10.4 Contagem de mesófilos, psicrotróficos e bolores e leveduras

A contagem total de aeróbios mesófilos, aeróbios psicrotróficos, bolores e leveduras em amostras de microgreens armazenados em embalagens abertas, seladas e sob atmosfera modificada pelo período de 0, 5 e 10 dias foi realizada segundo os métodos de plaqueamento APHA 08:2015, APHA 13.61:2015 e APHA 21:2015, respectivamente.

Para isso, 25 g da amostra foram homogeneizadas em 225 mL de água peptonada 0,1% (diluição 10^{-1}). A partir dessa primeira diluição foram preparadas diluições decimais seriadas da amostra (até 10^{-7}). Em seguida, foram selecionadas quatro diluições adequadas de cada amostra e de cada diluição foi inoculado 100 µL em placas contendo Ágar Padrão de Contagem (PCA) para mesófilos e psicrotróficos e placas de Ágar

Dicloran Rosa de Bengala Cloranfenicol (DRBC) para bolores e leveduras. Posteriormente, as placas foram incubadas a 35 ºC por 48 h para contagem de aeróbios mesófilos, 7 ºC por 10 dias para contagem de aeróbios psicrotróficos e 25 ºC por 5 dias para contagem de bolores e leveduras (Silva et al., 2021). Os resultados foram expressos em log UFC/g.

4.11 Análises estatísticas

Os parâmetros de composição centesimal, vitamina C, quantificação dos teores de compostos bioativos, assim como atividade antioxidante e as análises microbiológicas nos vegetais avaliados foram realizados com delineamento inteiramente casualizado, em triplicata e os resultados foram expressos em média ± desvio padrão. A análise estatística utilizada foi ANOVA one-way, já os dados referentes à vida útil nas diferentes embalagens foram submetidos à ANOVA two-way. Ambas com teste de comparação das médias pelo teste de Tukey ao nível de 5 % de significância, assim, em todos os casos foram consideradas diferenças significativas quando $p \leq 0,05$. Todas as análises estatísticas foram realizadas utilizando o software Statistica (versão 14.0.1).

CAPÍTULO 4

5. RESULTADOS

Os resultados deste trabalho estão divididos em três artigos apresentados a seguir, sendo um de revisão e dois experimentais.

O artigo 1, intitulado “Overview on bioactive compounds’ profile of Brassicaceae microgreens: An approach on different production systems and the use of elicitors’’, trata de uma revisão bibliográfica sobre microgreens da família Brassicaceae, considerando os diferentes sistemas de cultivo e o uso de elicitores, e foi publicado na revista *Acta Botanica Brasilica*.

O artigo 2, intitulado “Nutritional quality and bioactive compouns of arugula (*Eruca sativa* L.) sprouts and microgreens’’, trata da comparação do perfil nutricional e bioativo de brotos e microgreens de rúcula e foi publicado na revista *International Journal of Food Science & Technology*.

O artigo 3, intitulado “Postharvest quality of arugula (*Eruca sativa*) microgreens determined by microbiological, physico-chemical, and sensory parameters’’, trata da análise de vida útil dos microgreens de rúcula armazenados em diferentes embalagens, e foi publicado na revista *Foods*.

5.1 ARTIGO 1 – "Visão geral do perfil de compostos bioativos dos microgreens de Brassicaceae: Uma abordagem sobre diferentes sistemas de produção e o uso de elicitores''

Abstract: We investigated the literature to find the bioactive compounds' profile of Brassicaceae microgreens and the influence of different production systems and the elicitors use in its overall quality. For this, a summary of the latest progress in bioactive compounds qualification and quantification are presented in the relevant databases. Determining the exact role of production systems is not a straightforward process, although it seems to have greater influence according to the intended plant. From the nutritional point of view, the microgreens production demonstrates a high content of bioactive compounds. The use of elicitors, as one of the dependent variables, appears to increase the concentrations of bioactive compounds, especially the use of the light.

Besides that, the conditions of growth, harvest and processing remain crucial factors that should be considered in the successful development of the seed.

Keywords: young leaves; phytochemical profile; brassicas; seed; growth conditions.

1. Introduction

Microgreens are young leafy vegetables, harvested when the first cotyledons expand completely and usually before the real leaves appear. This crop has a fast production cycle, from one to three weeks (Kopsell et al., 2012), and can be produced in greenhouses, in the soil or, more commonly, in soilless systems (Di Gioia et al., 2015). These characteristics demonstrate the potential of these leafy vegetables to adapt production to a smaller scale and, consequently, to spread their consumption more widely (Kyriacou et al., 2016).

In addition to being produced quickly, easily and economically due to the simple equipment and supplies requirements, microgreens also have an advantage from the sustainability perspective (Galieni et al., 2020): most cultures demand few resources, such as water or energy and no fertilizer, as the seed provides adequate nutrition for the plant (Xiao et al., 2015; Weber, 2017).

Is the Brassicaceae family, composed mainly of floral and leafy cruciferous vegetables, which is the most consumed plant family worldwide, due to its characteristic flavor and known functional properties, which are directly related to its phytochemical composition (Xiao et al., 2019). Several authors have reported that these vegetables are characterized by a higher concentration of bioactive compounds than those of the same species when harvested in the standard growth stage, and therefore could be considered

as a functional food (Xiao et al., 2012, Ebert et al, 2014; Mir et al., 2016; Verlinden, 2019).

Based on the literature, differences in the phytochemical's concentrations, which potentially produce healthy effects, can be founded when comparing young and mature parts of the same plant. In mature vegetables, the distribution of bioactive compounds may differ according to the specific part of the plant considered (Tomas et al., 2021). In the case of microgreens, they are still living tissues after harvest and continue their biological processes, such as transpiration and respiration (Liu et a., 2020). Furthermore, as the development of the vegetable epidermis is minimal in microgreens, the bioactive compounds bioavailability is higher than in mature stages (Choe et al., 2018).

Therefore, the aim of this work is investigating the literature to find the bioactive compounds' profile of Brassicaceae microgreens and the influence of different production systems and the use of elicitors in its overall quality.

2. Search strategy

This review was reported following the PRISMA recommendation (Aguiar et al., 2018). This study included articles from scientific journals that evaluated phytochemical composition of Brassicaceae microgreens over the past ten years in English, Portuguese and Spanish. Experimental studies that evaluated biochemically microgreens from the Brassicaceae family were added or any type of descriptive analysis on the subject were included (Figure 1). The following measures were applied as exclusion criteria: 1) patents, quotations, letters, conference abstracts, case reports; 2) studies that used only microgreens from another family; 3) studies which were not evaluated bioactive

compounds; 4) studies that used microgreens for the production of foods.

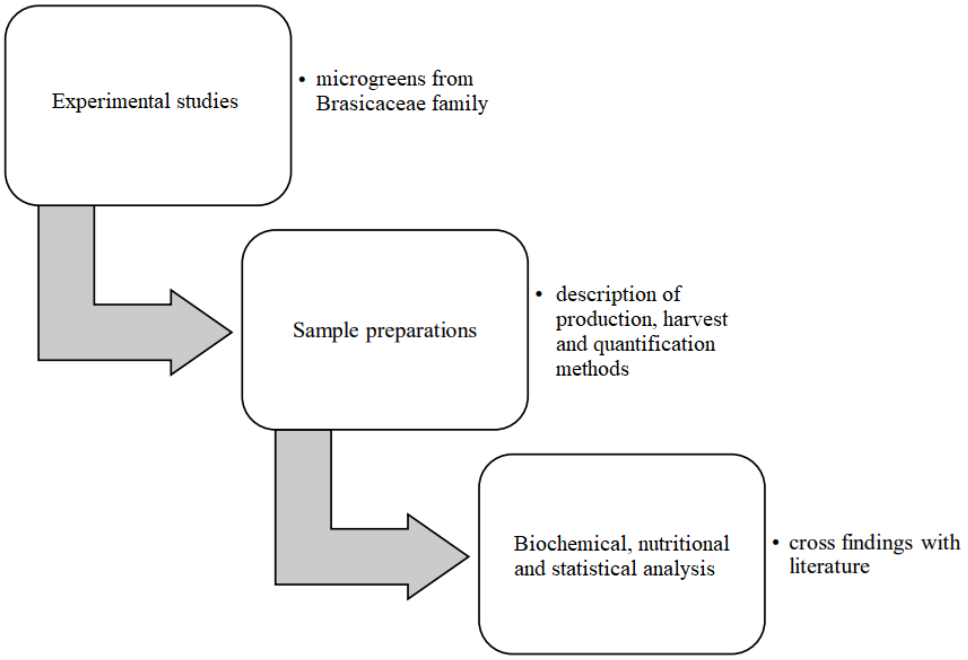


Figure 1. Organization of the study proposal for the quality assessment of Brassicaceae microgreens

Detailed individual search strategies were developed for each of the following databases: Food Science and Technology Abstracts (FSTA), Science Direct and Web of Science. Appropriate combinations of words were selected and adapted for research in each database. All references were managed by Mendeley desktop software version 1.17.11 and duplicate articles were removed.

The selection of the studies was completed in 2 steps (Figure 2). In step 1, two researchers independently identified the articles that followed the inclusion criteria and discarded the others. In step 2, the same reviewers checked the methodology of the articles. Finally, the articles that fulfilled the two steps were included. The reference list

of selected studies was critically evaluated by the reviewers. Any disagreement in the first or second phase was decided by discussion until agreement was reached between the reviewers.

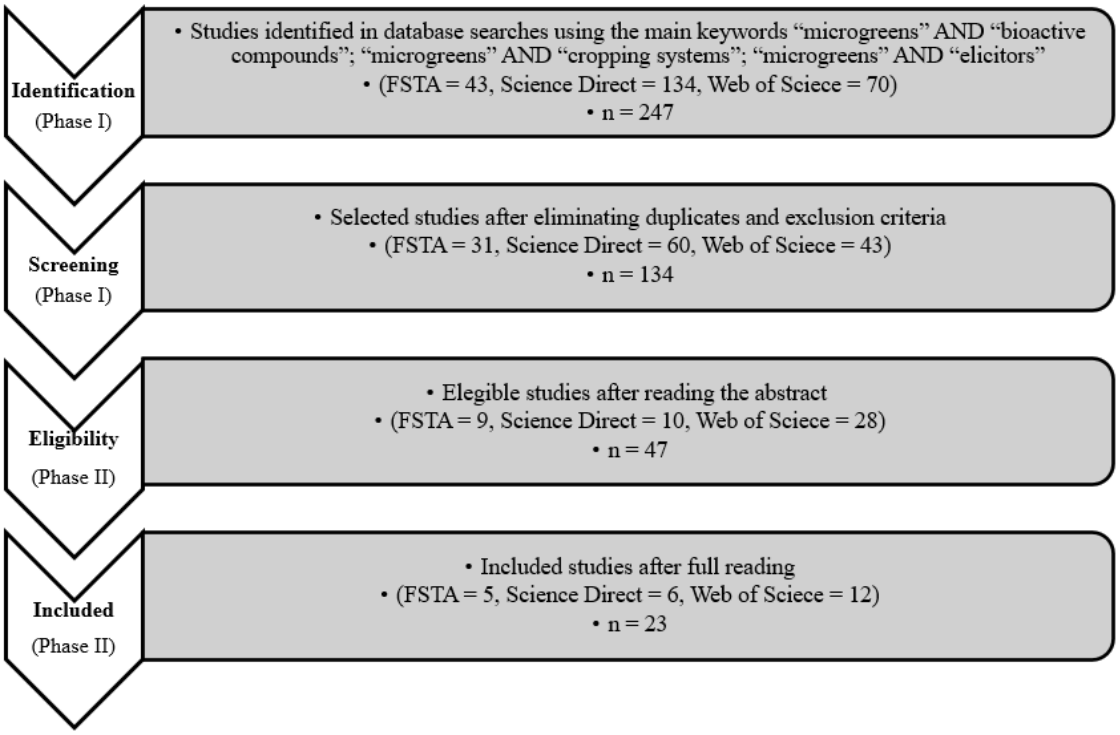


Figure 2. Flowchart of the selection of articles analyzed in this review

3. Influence of production systems and the use of elicitors

The microgreen's production is usually carried out in a controlled environment, inside greenhouses, using soilless cultivation systems (Di Gioia et al., 2017; Liu et al., 2020). Choosing a culture medium with adequate microbiological characteristics, as well as the humidity and insects' control, is extremely important to ensure a safe microgreens

consumption, as the chosen medium can represent a contamination source (Di Gioia et al., 2016).

Available to consumers in supermarket chains as well as on local farms, the microgreens growth environments are quite different: On a local farm, these vegetables are generally grown in the soil, while for the supermarket they are grown hydroponically, which increases productivity, but can compromise nutritional and sensory quality due to longer transport and storage (Tan et al., 2019).

In the study by Fortunã et al. (2018), there is a variation in the mineral content, depending on the production system. The main difference between these systems, with regard to mineral nutrition, is related to the soil matrix influence, which can change mineral availability.

Internal cultivation and greenhouse systems not only allow significantly higher yields (up to 30% increase) compared to open field systems, but can also facilitate off-season production and substantial chemical composition and bioactive profile manipulation of the final product. Vegetable production in a hydroponic crop appears to be an effective tool for increasing the phytochemicals content, according to the studies reviewed, as well as to control the antinutrients accumulation, such as nitrates (Rouphael et al., 2018). As reported by these authors, the combination of genotype, substrate and the environmental conditions management can maximize product quality in a controlled environment.

Compared to traditional soil cultivation, soilless cultivation systems offer the opportunity to standardize the production process in order to achieve faster growth, all year round and with greater efficiency in water and nutrients use. In addition, these systems provide the possibility to regulate secondary metabolism through adequate

composition and concentration control of the nutrient solution (Borgognone et al., 2016) and to adapt the production organically to the domestic scale (Kyriacou et al., 2016).

The influence of different agronomic practices or environmental stresses on secondary metabolites can be modified by the effects of potential others covariates, such as soil type, irrigation water, season of the year, temperature, insects, seed disinfection, handling and post-harvest procedures (Riggio et al., 2019). As the time between sowing and harvesting microgreens differs between species (Kyriacou et al., 2018), growers should select crops that have a similar growth rate so that the crop can be harvested all at once (Ebert et al., 2014).

According to Galieni et al. (2020), any stressing condition during germination can work as an elicitor, i.e., it may stimulate secondary metabolisms and increase the phytochemical content of microgreens. Thus, several studies aim to apply abiotic elicitors, such as LED light, and biotics, such as plant hormones, in order to expand, consequently, health benefits (Samuolienė et al., 2013; Renna et al., 2016; Franco et al., 2016; Baenas et al., 2019; Yadav et al., 2019; Ramirez et al., 2020).

One of the benefits of using light as an elicitor is the possibility of selecting different qualities and intensities that will act on the morphology of plants and, consequently, on the synthesis of phytochemicals (Craver et al., 2017). On the other hand, exposure to light during storage had no effect on α-tocopherol or total phenolic compounds concentrations, but accelerated the deterioration of sensory quality. Storage in the dark resulted in greater capacity for eliminating hydroxyl radicals and retaining carotenoids (Xiao et al., 2014). According to Brazaityté et al. (2015), the spectral quality of light regulation depends on the species and can change the content of bioactive compounds.

As for biofortification, the Brassicaceae species that grow in soilless systems are good candidates for producing mineral-fortified microgreens, when the nutrient solution composition is adjusted. This strategy depends on the appropriate crops' selection and the biofortification process standardization, in order to guarantee a high quality and safe vegetable for consumption (Di Gioia et al., 2019; Pannico et al., 2020).

In relation to fertilizers, they have been used for a long time to provide essential nutrients for plant growth. Murphy et al. (2010) found that calcium nitrate, ammonium nitrate and urea influenced a greater microgreens fresh weight. Sun et al. (2015) reported the potential effect of calcium chloride on the nutritional value of microgreens, when they verified an increase in the glucosinolates concentrations.

Among plant hormones, methyl-jasmonate has been applied to increase the bioactive compounds content (Zhu et al., 2019; Nuñez-Gómez et al., 2020). For Baenas et al. (2014), the effect of phytohormones throughout the germination with salicylic acid caused a 20% increase in the total of broccoli and radish glucosinolates. Phytohormones interact in the defense signaling genes expression, being accumulated after pathogenic or environmental stresses. The use of this type of elicitor is due to its ability to simulate the responses of the plant's defense, which lead to bioactive compounds production (Poulev et al., 2003).

In the last decade, the scientific literature on microgreens has increased. Studies published in recent years demonstrate the nutritional potential of these young plants that can be influenced by production systems and growth conditions for a successful harvest (figure 3). They also demonstrated that, instead of isolated supplementation of these

nutrients, the human body takes better advantage of the interactions of these phytochemicals in their different sources of origin (Liu, 2013; Choe et al., 2018).

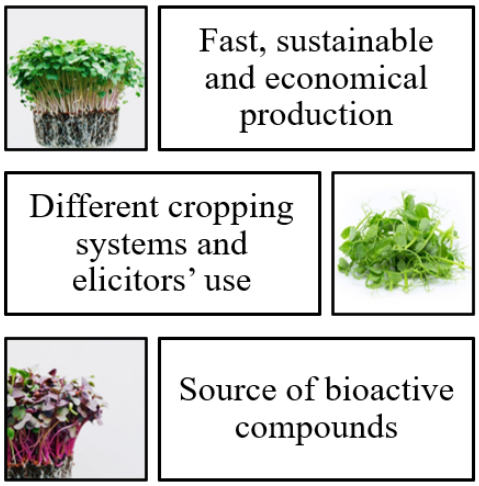


Figure 3. Highlights of microgreens' production

4. Characterization of bioactive compounds from microgreens

There are aspects that have been little explored about microgreens, as gathering information about the bioactive compounds' profile of whole Brassicaceae family and not just some members (Galieni et al., 2020). In this sense, Table 1 shows the secondary metabolites' characterization and quantification of this family.

Table 1. Characteristics of microgreens studies in the Brassicaceae family

Brassicaceae microgreens	Findings	Reference
Mustard (*Brassica juncea* L. Czern)	463 µmol m-2s-1 of light intensity resulted in an increased of β-carotene, neoxanthin and chlorophyll concentrations and a decreased of zeaxanthin and anteraxanthin.	Kopsell et al., 2012

Arugula (*Eruca sativa* Mill) China rose radish (*Raphanus sativus* L.) Green daikon radish (*Raphanus sativus* L. var. *longipinnatus*) Mizuna (*Brassica rapa* L. ssp. *nipposinica*) Opal radish (*Raphanus sativus* L.) Peppercress (*Lepidium bonariense* L.) Nutrient purple kohlrabi (*Brassica oleracea* L. var. *gongylodes*) Purple mustard (*Brassica juncea* L. Czern) Red cabbage (*Brassica oleracea* L. var. *capitata*) Red mustard (*Brassica juncea* L. *Czern*) Wasabi (*Wasabia japonica* Matsum)	Red cabbage and green daikon radish had the highest ascorbic acid, carotenoids, phylloquinones and tocopherols concentrations.	Xiao et al., 2012
Kohlrabi (*Brassica oleracea* var. *gongylodes*, 'Delicacy Purple') Mustard (*Brassica juncea* L., 'Red Lion') Red pak choi (*Brassica rapa* var. *chinensis*, 'Rubi F1') Tatsoi (*Brassica rapa* var. *rosularis*)	Intermediate light intensities (440 e 330 µmol m-2s-1) increased antioxidant capacity, anthocyanins and total phenolics and decreased nitrate levels.	Samuolienė et al., 2013
Daikon radish (*Raphanus sativus* var. *longipinnatus*)	Exposure to light during storage increased the ascorbic acid concentration. On the other hand, storage in the dark helped to preserve quality and prolong shelf life, with higher b-carotene, lutein/zeaxanthin levels and antioxidant activity. No significant differences in the α-tocopherol and total phenolics concentrations were found.	Xiao et al., 2014
Mustard (*Brassica juncea* L., *'Red Lion'*) Red pak choi (*Brassica rapa* var. *chinensis*, 'Rubi F1') Tatsoi (*Brassica rapa* var. *rosularis*)	Intermediate light intensities (440 e 330 µmol m-2s-1) increased the carotenoids content, especially α-carotene and lutein/zeaxanthin levels.	Brazaitytė et al., 2015
Broccoli (*Brassica oleracea* L. var. *italica*) Radish (*Raphanus sativus* cv. *rambo*)	The storage temperature influenced the quality and the bioactive compounds content. Storage at 5 °C is the most suitable. These crucifers remain acceptable for consumption after the 14-day storage period.	Baenas et al., 2017
Kohlrabi (*Brassica oleracea* var. *gongylodes*) Mustard (*Brassica juncea*, 'Garnet Giant') Mizuna (*Brassica rapa* var. *japonica*)	Increasing light intensity increased anthocyanin content and decreased carotenoid concentration. In addition, the light quality affected the chlorophyll and total phenolic concentrations.	Craver et al., 2017
Cress (*Lepidium sativum* cv. Curled) Kohlrabi (*Brassica oleracea* var. *gongylodes*) Komatsuna *(Brassica rapa var. perviridis)* Mibuna *(Brassica rapa var. laciniifolia*) Mustard (*Brassica juncea* L. Czern) Pak choi (*Brassica rapa* L.) Radish (*Raphanus sativus* L.) Tatsoi (*Brassica rapa* var. *rosularis*)	The bioactive compounds content, especially minerals, carotenoids, chlorophyll, antioxidant capacity and ascorbic acid, suffered variations among species.	Kyriacou et al., 2018
Mustard (*Brassica juncea* L. Czern) Leaf mustard *(Brassica juncea* subsp. *integrifolia)* Radish (*Raphanus sativus* L.) Cabbage (*Brassica oleracea L.* var. *capitata*)	On the 1st day of harvest, the species showed the highest total chlorophyll, ß-carotene, lycopene, ascorbic acid levels and the best antioxidant activity, while these substances	Polash et al., 2018

	deteriorated significantly on the 3rd or the 5th day of harvest.	
Mustard (*Brassica juncea* L. Czern)	The total phenolic, minerals and α-tocopherol content increased mainly under UV-A 402 nm, while the nitrate level increased under UV-A 366 and 390 nm. The lutein/zeaxanthin and β-carotene concentrations increased regardless of the wavelength and the time of exposure to light.	Brazaitytė et al., 2019
Broccoli (*Brassica oleracea* L. var. *italica* Plenck) Green curly kale (*Brassica oleracea* var. *sabellica* L.) Red mustard (*Brassica juncea* L. Czern) Radish (*Raphanus sativus* L.)	Compared to their mature counterparts, microgreens are, in general, good minerals and antioxidant sources. They also contain relevant ascorbic acid content and carotenoids levels.	De La Fuente et al., 2019
Mizuna (*Brassica rapa* var. *japonica* cv. Greens) Cress (*Lepidium sativum* cv. Curled)	In general, blue light increased the mineral content of microgreens, with variations among species. Monochromatic lights generated more quantifications of phenolic compounds and total phenolic in mizuna. But dichromatic light increased antioxidant capacity and lutein/zeaxanthin levels in cress. Variations in chlorophyll content within the Brassicaceae family were found. Probably due to differences in pigmentation in the microgreens leaves	Kyriacou et al., 2019
Kohlrabi (*Brassica oleracea* var. *gongylodes*) Broccoli (*Brassica oleracea*) Mizuna (*Brassica rapa* var. *japonica*)	The different qualities of light and wavelengths influenced the concentration of ascorbic acid and the β-carotene among the species.	Samuolienė et al., 2019
Broccoli (*Brassica oleracea* L.)	Microgreens from local farm had higher levels of chlorophyll and ascorbic acid. No significant difference in total phenolic concentration and the antioxidant capacity was found independent of the cultivation system.	Tan et al., 2019
Arugula (*Eruca sativa* Mill) Broccoli (*Brassica oleracea* L. var. *italica*) Brussel sprouts (*Brassica oleracea* L. var. g*emmifera*) Cabbage chinese (*Brassica rapa* L. var. *pekinensis*) Cabbage green (*Brassica oleracea* L. var. *capitata f. alba*) Cabbage red (*Brassica oleracea* L. var. *capitata f. rubra*) Cabbage savoy (*Brassica oleracea* L. var. *capitata f. sabauda*) Cauliflower (*Brassica oleracea* L. var. *botrytis*) Collard (*Brassica oleracea* L. var. *viridis*) Kale chinese (*Brassica oleracea* L. var. *alboglabra*) Kale red (*Brassica oleracea* L. var. *acephala*) Kale Tucsan (*Brassica oleracea* L. var. *acephala*)	The phytochemicals content and composition varied significantly among and within species. But, Brassicaceae microgreens are good sources of antioxidant phytochemicals. The main carotenoids found in these 30 samples of Brassicaceae microgreens were β-carotene, lutein/zeaxanthin and violaxanthin, with concentrations that varied 2.3, 7.9 and 5.5 times, respectively.	Xiao et al., 2019

Kohlrabi purple (*Brassica oleracea* L. var. *gongylodes*) Komatsuna red (*Brassica rapa* L. var. *perviridis*) Mizuna (*Brassica rapa* L. var. *nipposinica*) Mustard Dijon (*Brassica juncea* L. Czern) Mustard red (*Brassica juncea* L. Czern.) Pak choi (*Brassica rapa* L. var. *chinensis*) Peppercress (*Lepidium bonariense*) Radish China rose *(Raphanus sativus* L.) Radish daikon (*Raphanus sativus* L. var. *longipinnatus*) Radish red (*Raphanus sativus* L.) Radish ruby (*Raphanus sativus* L.) Rapini (*Brassica rapa L. var. ruvo*) Rutabaga (*Brassica napus L. var. napobrassica)* Tatsoi (*Brassica narinosa L. var. rosularis)* Turnip (B*rassica rapa L. var. rapa)* Upland cress (*Barbarea verna (P. Mill.) Aschers)* Wasabi (*Wasabia japonica* Matsum.) Watercress (*Nasturtium officinale L.)*		
Kohlrabi (*Brassica oleracea* var. *gongylodes*) Pak choi (*Brassica rapa* L. subsp. *chinensis*)	Natural fiber substrates, especially peat, had an increased nitrate and minerals concentration compared to synthetic. The chlorophylls, carotenoids and ascorbic acid concentrations were mainly influenced by the species. The variability in the polyphenol content was greater between species (8.85–14.33 $mg/kg^{-1}\cdot fw$) than between substrates (11.16–13.13 $mg/kg^{-1}\cdot fw$).	Kyriacou et al., 2020
Broccoli (*Brassica oleracea* var. *italica*) Broccoli raab (*Brassica oleracea* var. *botrytis*) Cauliflower (*Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort)	Cauliflower had the highest content of some mineral elements and α-tocopherol.	Palmitessa et al., 2020
Tatsoi (*Brassica rapa* L. subsp. *narinosa*)	The ideal Se dose that guarantees the biofortification effectiveness and improves the bioactive compounds content was 16 μM.	Pannico et al., 2020
Broccoli (*Brassica oleracea* var. *italica*) Broccoli raab (*Brassica oleracea* var. *botrytis*) Cauliflower (*Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort)	Microgreens showed a higher Nutrient Quality Score (NQS) than their mature counterpart, with emphasis on the cauliflower microgreens score, which was about six times higher. Effectiveness of the NQS in distinguishing differences in general nutritional quality terms, not only between different cultivation conditions, but also when comparing genotypes.	Renna et al., 2020
Arugula (*Diplotaxis tenuifolia* (Wild Rocket Napoli)) Cabbage (*Brassica oleracea* var. *capitata* (Green Cabbage Copenhagen)) Brussels sprouts (*Brassica oleracea* var. *gemmifera* (Green Brussels sprouts Mezzo Nano))	The absence of nutritional supplementation did not increase the content of bioactive compounds in brussels sprouts, but for cabbage microgreens yes, with an increase in total ascorbic acid and anthocyanins. For arugula, there was an increase in the carotenoids, total ascorbic acid and	El-Nakhel et al., 2021

	anthocyanins levels, but caused a decrease in total phenolic acids.	
Arugula (*Eruca sativa* (L.) Cav.) Broccoli (*Brassica oleracea* L. var. italica) Red cabbage (*Brassica oleracea* L. var. capitata)	Broccoli microgreens had more than twice the number of bioactive compounds than their mature counterpart, most of which consisted of lipids, phenolic compounds and alkaloids.	Johnson et al., 2021
Brócolis (*Brassica oleracea* L.), Daikon (*Raphanus raphanistrum* subsp. *sativus* (L.) Domin), Mustard (*Brassica juncea* (L.) Czern.) Rocket (*Eruca vesicaria* (L.) Cav.) Watercress (*Nasturtium officinale* R. Br.)	Broccoli microgreens showed the highest polyphenols, carotenoids and chlorophyll levels, in addition to good antioxidant capacity. Mustard was characterized by a high ascorbic acid and total sugar content. In contrast, the rocket microgreens exhibited the least antioxidant activity.	Marchioni et al., 2021
Kale (*Brassica oleracea L*. var. *acephala)* Kohlrabi (*Brassica oleracea L*. var. *gongylodes*) Cabbage (*Brassica oleracea L*. var. *capitata*) Radish (*Raphanus sativus* L.)	470 phytochemicals were found in the four microgreens of Brassicaceae. Among polyphenols, flavonoids were the most represented class. Glucosinolate bioaccessibility differed significantly between species.	Tomas et al., 2021

The contribution of microgreens to health can be attributed to their antioxidant capacity, in addition to a wide range of nutrients and bioactive components, such as: vitamins (mainly K, C and E), carotenoids, polyphenols and glucosinolates (Choe et al., 2018). The bioactive compounds present in the microgreens are variable and influenced by the growth conditions, harvest and processing (Sun et al., 2013; Argento et al., 2019). To compare profiles of a compound from the same family, cultivation and extraction and detection techniques should be considered (Ramirez et al., 2020).

The divergences found in the bioactive compounds' concentrations between microgreens and their mature counterparts are due to two main reasons: 1) microgreens do not undergo post-harvest treatments, therefore they do not undergo nutrient degradation and 2) the germination stage, in which physiological, biochemical and nutritional changes occur, due to the activation of enzymes (Choe et al., 2018; Yadav et al., 2019; Di Gioia et al., 2016; Liu et al., 2020).

During germination, some reserve materials of the seeds are degraded and used for respiration and synthesis of new cellular constituents in the developing embryo, causing significant alterations in the biochemical, nutritional and sensory characteristics (López-Amorós et al., 2006; Zhang et al. al., 2015). The activation of proteases, which help in the metabolization of proteins, increasing the bioavailability of nutrients, and other changes contribute to the increase in the metabolic activity of the seeds and, consequently, the increase in the bioactive compounds' concentration (Sibian et al., 2017).

Regarding the high respiratory and metabolic activity found in tissues with rapid growth and differentiation of microgreens, even minimal differences in the ontogeny stages in the harvest can detain disparate states of transient phenylpropanoid components, thus introducing qualitative variation in polyphenolic profiles, as well as affect its bioavailability and antioxidant potential (Dhuique-Mayer et al., 2009; Kyriacou et al., 2016; Ebert et al., 2017).

4.1. Leafy Vegetables

This section is focused on results found for leafy vegetables of Brassicaceae family about their phytochemical content. It was found that mustard was the most studied microgreen by the authors so far.

In the study by El-Nakhel et al. (2021), the nutrient supplementation absence elicited an extensive increase in secondary metabolite of arugula, as lutein (110%), β-carotene (30%), the total ascorbic acid (58%) and anthocyanins (20%), but it caused a decrease in total phenolic acids. According to these authors, the microgreens cultivation on a

commercial peat-based substrate without nutrient supplementation may be feasible for certain species.

For De la Fuente et al. (2019), mustard obtained the highest value of total anthocyanin content (36.4 mg of cyanidin-3-glycoside/100 g·dw), with statistical difference for the other evaluated vegetables. As for the soluble polyphenols content in the bioaccessible fraction, the lowest amount was observed in mustard (821 mg/100 g·dw). According to the authors, this decrease may be due to the slightly alkaline conditions reached after the intestinal phase, together with possible interactions with digestive enzymes.

Polash et al. (2018) demonstrated that the mustard microgreens showed the maximum of bioactive substances such as total chlorophyll (8.22 mg/100 g), ß-carotene (2.41 mg/100 g), lycopene (4.37 mg/100 g), ascorbic acid (16.23 mg/100 g) and antioxidant activity (DPPH) (0.75 µg/mL) on the harvest first day. The authors conclude that the microgreens consumption immediately after harvest is the best time to obtain the expected health benefits.

An explanation for the bioactive substances' degradation would be the need for an adequate minerals supply, water and light influx, responsible for various physiological and biochemical reactions and for maintaining the plants enzymatic activity. If any of these variables are not met, physiological and biochemical reactions end up leading to no production and/or degradation of these compounds in an attempt to survive. Instead of performing photosynthesis, the harvested microgreens start to produce toxic pigments and reactive oxygen species (Polash et al., 2018).

Regarding antioxidant activity, for Kyriacou et al. (2019) both lipophilic and hydrophilic activity, showed higher value in Brassicaceae species. According to the researchers, combined blue light is generally more effective than monochromatic blue or red light in increasing the lipophilic antioxidant capacity of most species (Marchioni et al., 2021). The photoreceptors combined activation by LED lights would be able to influence the enzymatic activities regulation responsible for the secondary metabolites' biosynthesis (Alrifai et al., 2019).

4.2. Floral Vegetables

Kohlrabi and cabbage and their varieties were the most studied microgreens by the authors of this review. His findings for these and other floral vegetables from the Brassicaceae family on bioactive compounds are described below.

In relation to the antioxidant capacity, for Tomas et al. (2021), radish purple microgreens showed increased antioxidant activity by the CUPRAC (6694.2 mg TE/100 g·dw) method, with a statistically significant difference for kohlrabi and red cabbage. For De la Fuente et al. (2019), the radish showed higher total content (488.65 µM Trolox Eq/100 g) and higher bioaccessible fraction (137.70 µM Trolox Eq/100 g), this, with a significant difference, using the TEAC method.

The differences between the methodologies may be related to the compounds formed after the digestion process, which are susceptible to various reactions with substrates and free radicals according to each antioxidant method, depending on the matrix. The

decrease in antioxidant capacity observed in both methods after digestion in vitro is attributable to the bioactive compounds' reduction (De La Fuente et al., 2019).

Tan et al. (2019) evaluated the bioactive compounds of broccoli microgreens grown by different methods (hydroponically vs. soil cultivation) and from different sources (commercial vs. local farm). A significantly higher chlorophyll concentration was found in hydroponic system and in the soil (0.33 and 0.30 mg/g, respectively) compared to commercial one (0.029 mg/g).

The explanation for this difference is that commercial samples may have been taken before the cotyledon leaves development, where chlorophyll accumulates and/or chlorophyll may have been degraded due to the long supply chain and the storage time, deteriorating the vegetable freshness. The result for the total chlorophyll content was fifteen times higher than the stipulated for mature broccoli (Tan et al., 2019).

5. Conclusions

While determining the exact production systems role is not a straightforward process, although it seems to have greater influence according to the intended plant, from a biochemical point of view, the microgreens production demonstrates a high bioactive compounds content and a good source of food health for human diet. The use of elicitors, mostly artificial light, as one of the dependent variables, appear to increase the concentrations of bioactive compounds. Furthermore, it was evident that, more than the family or even the species, it is the seed genotype and the conditions of growth, harvest and processing that will determine the plantation success. For the dissemination of its

consumption as a viable vegetable alternative, it is necessary to understand these mechanisms, in order to improve its production technique.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

References

Alrifai, O., Hao, X., Marcone, M. F., & Tsao, R. (2019). Current review of the modulatory effects of LED lights on photosynthesis of secondary metabolites and future perspectives of microgreen vegetables. *Journal of Agricultural and Food Chemistry, 67*, 6075–6090.

Argento, S., Melilli, M., & Branca, F. (2019). Enhancing Greenhouse Tomato-Crop Productivity by Using *Brassica macrocarpa* Guss. Leaves for Controlling Root-Knot Nematodes. *Agronomy, 9,* 820-833.

Baenas, N., García-Viguera, C., & Moreno, D. (2014). Biotic Elicitors Effectively Increase the Glucosinolates Content in Brassicaceae Sprouts. *Journal of Agricultural and Food Chemistry, 62(8),* 1881–1889.

Baenas, N., Gómez-Jodar, I., Moreno, D. A., García-Viguera, C., & Periago, P. M. (2017). Broccoli and radish sprouts are safe and rich in bioactive phytochemicals. *Postharvest Biology and Technology, 127, 60–67.*

Baenas, N., Fusari, C., Moreno, D. A., Valero, D., & García-Viguera, C. (2019). Biostimulation of bioactive compounds in radish sprouts (Raphanus sativus

"Rambo") by priming seeds and spray treatments with elicitors. *Acta Horticulturae, 1256,* 659–663.

Borgognone, D., Rouphael, Y., Cardarelli, M., Lucini, L., & Colla, G. (2016). Changes in biomass, mineral composition, and quality of cardoon in response to NO_3^-:Cl^- ratio and nitrate deprivation from the nutrient solution. *Frontiers in Plant Science, 7*, 978-987.

Brazaitytė, A., Sakalauskienė, S., Samuolienė, G., Jankauskienė, J., Viršilė, A., Novičkovas, A., & Duchovskis, P. (2015). The effects of LED illumination spectra and intensity on carotenoid content in Brassicaceae microgreens. *Food Chemistry, 173,* 600–606.

Brazaitytė, A., Viršilė, A., Samuolienė, G., Vaštakaitė-Kairienė, V., Jankauskienė, J., Miliauskienė, J., & Duchovskis, P. (2019). Response of Mustard Microgreens to Different Wavelengths and Durations of UV-A LEDs. *Frontiers in Plant Science, 10,* 1153-1164.

Choe, U., Yu, L. & Wang, T. (2018). The science behind *microgreens* as an exciting new food for the 21th century. *Journal of Agricultural and Food Chemistry, 66*, 11519-11530.

Craver, J., Gerovac, J., Lopez, R., & Kopsell, D. (2017). Light Intensity and Light Quality from Sole-source Light-emitting Diodes Impact Phytochemical Concentrations within Brassica Microgreens. *Journal of the American Society for Horticultural Science, 142(1),* 3–12.

De la Fuente, B., López-García, G., Mañez, V., Alegría, A., Barberá, R., & Cilla, A. (2019). Evaluation of the Bioaccessibility of Antioxidant Bioactive Compounds and Minerals of Four Genotypes of Brassicaceae Microgreens. *Foods, 8(7),* 250-266.

Di Gioia F, Mininni C & Santamaria P. (2015). How to grow *microgreens*, in *Microgreens*. (pp. 51–79). Ed.:Di Gioia F and Santamaria P. Eco-logica, Bari.

Di Gioia, F., De Bellis, P., Mininni, C., Santamaria, P., & Serio, F. (2016). Physicochemical, agronomical and microbiological evaluation of alternative growing media for the production of rapini (Brassica rapa L.) microgreens. *Journal of the Science of Food and Agriculture, 97(4),* 1212–1219.

Di Gioia, F., Renna, M. and Santamaria, P. (2017). Sprouts, *Microgreens* and "Baby Leaf" Vegetables. In: *Food Engineering Series, pp.* 403–432.

Di Gioia, F., Petropoulos, S., Ozores-Hampton, M., Morgan, K., & Rosskopf, E. (2019). Zinc and Iron Agronomic Biofortification of Brassicaceae Microgreens. *Agronomy, 9(677),* 677-697.

Dhuique-Mayer, C., Fanciullino, A.-L., Dubois, C., & Ollitrault, P. (2009). Effect of Genotype and Environment on Citrus Juice Carotenoid Content. *Journal of Agricultural and Food Chemistry, 57(19),* 9160–9168.

Ebert, A., Wu, T., & Yang, R. Y. (2014). Amaranth sprouts and microgreens—A homestead vegetable production option to enhance food and nutrition security in the rural–urban continuum. In: *Proceedings of the regional symposium on sustaining small-scale vegetable production and marketing systems for food and nutrition security.* (pp. 233–244). Taiwan: AVRDC Publication.

Ebert, A., Chang, C., Yan, M., & Yang, R. (2017). Nutritional composition of mungbean and soybean sprouts compared to their adult growth stage. *Food Chemistry, 237*, 15-22.

El-Nakhel, C., Pannico, A., Graziani, G., Kyriacou, M., Gaspari, A., Ritieni, A., De Pascale, S., & Rouphael, Y. (2021). Nutrient Supplementation Configures the Bioactive Profile and Production Characteristics of three Brassica Microgreens Species Grown in Peat-Based Media. *Agronomy, 11*, 346-357.

Fortună, M.-E., Vasilache, V., Ignat, M., Silion, M., Vicol, T., Patraș, X., & Lobiuc, A. (2018). Elemental and macromolecular modifications in *Triticum aestivum* L. plantlets under different cultivation conditions. *PLOS ONE, 13(8)*, e0202441.

Franco, P., Spinozzi, S., Pagnotta, E., Lazzeri, L., Ugolini, L., Camborata, C., & Roda, A. (2016). Development of a liquid chromatography–electrospray ionization–tandem mass spectrometry method for the simultaneous analysis of intact glucosinolates and isothiocyanates in Brassicaceae seeds and functional foods. *Journal of Chromatography, 1428*, 154–161.

Galieni, A., Falcinelli, B., Stagnari, F., Datti, A., & Benincasa, P. (2020). Sprouts and microgreens: trends, opportunities, and horizons for novel research. *Agronomy*, *10*, 1424-1469.

Johnson, S., Prenni, J., Heuberger, A., Isweiri, H., Chaparro, J., Newman, S., Uchanski, M., Omerigic, H., Michell, K., Bunning, M., Foster, M., Thompson, H., & Weir, T. (2021). Comprehensive evaluation of metabolites and minerals in 6 microgreen species and the influence of maturity. *Current developments in nutrition, 5(2)*, nzaa180.

Kopsell, D., Pantanizopoulos, N., Sams, C., & Kopsell, D. (2012). Shoot tissue pigment levels increase in “Florida Broadleaf” mustard (Brassica juncea L.) microgreens following high light treatment. *Scientia Horticulturae, 140,* 96–99.

Kyriacou, M., Rouphael, Y. Di Gioia, F., Kyratzis, A., Serio, F., Renna, M., & de Pascale, S. (2016). Micro-scale vegetable production and the rise of microgreens. *Trends in Food Science and Technology, 57,* 103–115.

Kyriacou, M. C., El-Nakhel, C., Graziani, G., Pannico, A., Soteriou, G. A., Giordano, M., & Rouphael, Y. (2018). Functional quality in novel food sources: genotypic variation in the nutritive and phytochemical composition of thirteen microgreens species. *Food Chemistry, 277,* 107-118.

Kyriacou, M., El-Nakhel, C., Pannico, A., Graziani, G., Soteriou, G. A., Giordano, M. & Rouphael, Y. (2019). Genotype-Specific Modulatory Effects of Select Spectral Bandwidths on the Nutritive and Phytochemical Composition of Microgreens. *Frontiers in Plant Science, 10,* 1501-1517.

Kyriacou, M., El-Nakhel, C., Pannico, A., Graziani, G., Soteriou, G., Giordano, M., Palladino, M., Ritieni, A., De Pascale, S. & Rouphael, Y. (2020). Phenolic Constitution, Phytochemical and Macronutrient Content in Three Species of Microgreens as Modulated by Natural Fiber and Synthetic Substrates. *Antioxidants, 9(252),*1-23.

Liu, R. H. (2013). Dietary Bioactive Compounds and Their Health Implications. Journal of Food Science, 78(s1), A18–A25.

Liu, W., Zhang, M., & Bhandari, B. (2020). Nanotechnology–A shelf life extension strategy for fruits and vegetables. *Critical Reviews in Food Science and Nutrition, 10*, 1706-1721.

López-Amorós, M. L., Hernández, T., & Estrella, I. (2006). Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal of Food Composition and Analysis*, *19*(4), 277-283.

Marchioni, I., Martinelli, M., Ascrizzi, R., Gabbrielli, C., Flamini, G., Pistelli, L., & Pistelli, L. (2021). Small functional foods: Comparative phytochemical and nutritional analyses of five microgreens of the Brassicaceae Family. *Foods, 10(2),* 427-438.

Mir, S. A., Shah, M. A., & Mir, M. M. (2016). *Microgreens*: Production, shelf life, and bioactive components. *Critical Reviews in Food Science and Nutrition, 57(12),* 2730–2736.

Murphy, C., Llort, K., & Pill, W. G. (2010). Factors Affecting the Growth of Microgreen Table Beet. *International Journal of Vegetable Science, 16(3),* 253–266.

Nuñez-Gómez, V., Baenas, N., Navarro-González, I., García-Alonso, J., Moreno, D. A., González-Barrio, R., & Periago-Castón, M. J. (2020). Seasonal Variation of Health-Promoting Bioactives in Broccoli and Methyl-Jasmonate Pre-Harvest Treatments to Enhance Their Contents. *Foods, 9(10),* 1371 – 1389.

Palmitessa, O. D., Renna, M., Crupi, P., Lovece, A., Corbo, F., & Santamaria, P. (2020). Yield and Quality Characteristics of Brassica Microgreens as Affected by the NH4:NO3 Molar Ratio and Strength of the Nutrient Solution. *Foods, 9(5)*, 677-694.

Pannico, A., El-Nakhel, C., Graziani, G., Kyriacou, M. C., Giordano, M., Soteriou, G. A., & Rouphael, Y. (2020). Selenium Biofortification Impacts the Nutritive Value,

Polyphenolic Content, and Bioactive Constitution of Variable Microgreens Genotypes. *Antioxidants, 9(4),* 272-294.

Polash, M., Sakil, M., & Hossain, M. (2018). Post-harvest biodegradation of bioactive substances and antioxidant activity in microgreens. *Journal of the Bangladesh Agricultural University*, *16*(2), 250-253.

Poulev, A., O'Neal, J., Logendra, S., Pouleva, R., Timeva, V., Garvey, A., Gleba, D., Jenkins, I., Halpern, B., Kneer, R., Cragg, G., & Raskin, I. (2003) Elicitation, a new window into plant chemodiversity and phytochemical drug discovery. *Journal of Medicinal Chemistry, 6,* 2542−2547.

Ramirez, D, Abellán-Victorio, A, Beretta, V, Camargo, A & Moreno, DA. (2020) Functional Ingredients From Brassicaceae Species: Overview and Perspectives. *International Journal of Molecular Sciences*, 21: 1998.

Renna, M, Di Gioia, F, Leoni, B, Mininni C & Santamaria P. (2016) Culinary Assessment of Self-Produced Microgreens as Basic Ingredients in Sweet and Savory Dishes. *Journal of Culinary Science & Technology,* 15: 126–142.

Renna, M., Stellacci, A., Corbo, F., & Santamaria, P. (2020). The Use of a Nutrient Quality Score is Effective to Assess the Overall Nutritional Value of Three Brassica Microgreens. *Foods, 9(9),* 1226-1241.

Riggio, G., Wang, Q., Kniel, K., & Gibson, K. (2019). Microgreens—A review of food safety considerations along the farm to fork continuum. *International Journal of Food Microbiology*, *290*, 76-85.

Rouphael, Y., Kyriacou, M. C., Petropoulos, S. A., De Pascale, S., & Colla, G. (2018). Improving vegetable quality in controlled environments. *Scientia Horticulturae 234*, 275–289.

Samuolienė, G., Brazaitytė, A., Jankauskienė, J., Viršilė, A., Sirtautas, R., Novičkovas, A., & Duchovskis, P. (2013). LED irradiance level affects growth and nutritional quality of Brassica microgreens. *Open Life Sciences, 8(12),* 1241-1249.

Samuolienė, G., Brazaitytė, A., Viršilė, A., Miliauskienė, J., Vaštakaitė-Kairienė, V., & Duchovskis, P. (2019). Nutrient Levels in Brassicaceae Microgreens Increase Under Tailored Light-Emitting Diode Spectra. *Frontiers in Plant Science, 10,* 1475-1484.

Sibian, M. S., Saxena, D. C., & Riar, C. S. (2017). Effect of germination on chemical, functional and nutritional characteristics of wheat, brown rice and triticale: a comparative study. *Journal of the Science of Food and Agriculture*, *97*(13), 4643-4651.

Sun, J., Xiao, Z., Lin, L., Lester, G. E., Wang, Q., Harnly, J. M., & Chen, P. (2013). Profiling Polyphenols in Five Brassica Species Microgreens by UHPLC-PDA-ESI/HRMSn. *Journal of Agricultural and Food Chemistry, 61(46),* 10960–10970.

Sun, J., Kou, L., Geng, P., Huang, H., Yang, T., Luo, Y., & Chen, P. (2015). Metabolomic Assessment Reveals an Elevated Level of Glucosinolate Content in CaCl2 Treated Broccoli Microgreens. *Journal of Agricultural and Food Chemistry, 63(6),* 1863–1868.

Tan, L., Nuffer, H., Feng, J., Kwan, S. H., Chen, H., Tong, X., & Kong, L. (2019). Antioxidant Properties and Sensory Evaluation of Microgreens from Commercial and Local Farms. *Food Science and Human Wellness, 9(1),* 45-51.

Tomas, M., Zhang, L., Zengin, G., Rocchetti, G., Capanoglu, E., & Lucini, L. (2021). Metabolomic insight into the profile, in vitro bioaccessibility and bioactive properties of polyphenols and glucosinolates from four Brassicaceae microgreens, *Food Research International, 140,* 1-11.

Verlinden, S. Microgreens: Definitions, Product Types, and Production Practices. In *Horticultural Reviews,* 1e.; Ian Warrington; John Wiley & Sons, Inc, 2019; Volume 47, pp. 85-125.

Weber, C. F. (2017). Broccoli Microgreens: A Mineral-Rich Crop That Can Diversify Food Systems. *Frontiers in Nutrition, 4(7),* 1-9.

Xiao, Z., Lester, G. E., Luo, Y., & Wang, Q. (2012). Assessment of Vitamin and Carotenoid Concentrations of Emerging Food Products: Edible Microgreens. *Journal of Agricultural and Food Chemistry, 60(31),* 7644–7651.

Xiao, Z., Lester, G. E., Luo, Y., Xie, Z., Luo, Y., & Wang, Q. (2014). Effect of light exposure on sensorial quality, concentrations of bioactive compounds and antioxidant capacity of radish microgreens during low temperature storage. *Food Chemistry, 151,* 472–479.

Xiao, Z., Lester, G., Park, E., Saftner, R., Luo, Y., & Wang. Q. (2015). Evaluation and correlation of sensory attributes and chemical compositions of emerging fresh produce: Microgreens. *Postharvest Biology and Technology, 110,* 140-148.

Xiao, Z., Rausch, S., Luo, Y., Sun, J., Yu, L., Wang, Q., Chein, P., & Stommel, J. (2019). Microgreens of Brassicaceae: Genetic diversity of phytochemical concentrations and antioxidant capacity. *LWT,* 101, 731-737.

Yadav, L., Koley, T., & Tripathi, A. (2019). Antioxidant potentiality and mineral content of summer season leafy greens: Comparison at mature and microgreen stages using chemometric. *Agricultural Research, 8,* 165-175.

Zhang, G., Xu, Z., Gao, Y., Huang, X., Zou, Y., Yang, T. (2015). Effects of Germination on the Nutritional Properties, Phenolic Profiles, and Antioxidant Activities of Buckwheat. Journal of Food Science. 2015, 80, H1111–H1119.

Zhu, Y., Wang, F., & Guo, L. (2019). Effect of jasmonic acid on glucosinolate metabolism in different organs of broccoli sprouts. *Emirates Journal of Food and Agriculture, 31(2),* 81-87.

5.2 ARTIGO 2 – "Qualidade nutricional e compostos bioativos de brotos e microgreens de rúcula (*Eruca sativa* L.)''

Original article

Nutritional quality and bioactive compounds of arugula (*Eruca sativa* L.) sprouts and microgreens

Marina Rocha Komeroski, Keyla Araujo Portal, Jorgiana Comiotto, Tâmmila Venzke Klug, Simone Hickmann Flores, Alessandro de Oliveira Rios

First published: 01 August 2023 | https://doi.org/10.1111/ijfs.16607

Abstract: As an alternative to vegetables in their traditional stages of maturation, sprouts, and microgreens are increasing in popularity mainly due to the potential health benefit of the contents of bioactive compounds. This work aimed to evaluate and compare the nutritional profile and the bioactive compounds of arugula (*Eruca sativa* L.) sprouts and microgreens produced organically in a hydroponic system. Seeds were placed in phenolic foams and soaked in water for 48 h to promote germination according to the supplier's recommendation. Sown trays were placed on top of perforated polystyrene boards and irrigated using a nutrient solution. Seeded trays were kept in a controlled environment and were exposed to 12 h photoperiod. Sprouts were harvested after 7 days of germination, while the microgreens were harvested after 14. As a result, these vegetables showed a high protein content, with sprouts being statistically different from microgreens (24.8% sprouts; 18.2% microgreens), with an excellent content of total fiber (34.3% sprouts; 28.7% microgreens) and soluble fiber (11.5% sprouts; 6.7% microgreens). Regarding the bioactive compounds, the predominance of carotenoids zeaxanthin and β-carotene in the sprouts (41.8 and 19.8μg/g, respectively) and the identification of more compounds as O-glucosides derived from flavonoids in the microgreens were noted.

Keywords: functional food; phytochemicals; young leaves; rocket; composition.

1. INTRODUCTION

Microgreens is a commercial term for immature plants, intermediate in size between sprouts and baby greens, which are harvested after the development of the first cotyledons, usually between 7-21 days after sowing (Di Gioia et al., 2017; Choe et al., 2018). Sprouts are characterized by roots originating from germinated seeds (Di Gioia et al., 2017), with regulated production (Ebert et al., 2014). The production characteristics of these plants make them ideal for production on a domestic (Katsenios et al., 2021), industrial, and even spatial scale (Kyriacou et al., 2017) since they allow food production in an enclosed space, with controlled environmental conditions and optimizing the use of natural resources and horticultural inputs (Katsenios et al., 2021).

Some cultivation conditions can afford a better bioactive quality for the horticultural commodities. In this sense, the applications of adequate light quality and quantity, temperature control, and nutrient use can enhance the content of bioactive compounds in sprouts and microgreens (Shah et al., 2017) since various enzymatic and non-enzymatic antioxidant compounds can be accumulatebe accumulated as a defense mechanism (Oh & Rajashekar, 2009).

According to O'Hare et al. (2007), germinated seeds may contain 2 to 10 times more phytochemicals depending on the species, cultivar, environmental conditions, and germination time. In this line, the analysis of the nutritional quality of microgreens evaluated by Ghoora et al. (2020a) and Xiao et al. (2012) also demonstrated higher phytochemical concentrations in relation to the value found in mature leaves. According to Johnson et al. (2021), this fact is largely driven by lipid and phenolic compounds, alkaloids, organosulfur compounds, vitamins, and amines, and the germination step that precedes them.

Sprouts and microgreens are considered sources of dietary fiber and vitamins such as A, C, and E besides β-carotene (Xiao et al., 2012; Ghoora et al., 2020). In addition, their nutritional composition includes functional compounds such as phenolics, carotenoids, among others (Zhang et al., 2020). These bioactive compounds have potential against chronic non-communicable diseases such as obesity, diabetes, and cardiovascular complications (Choe et al., 2018, Wojdylo et al., 2020).

The nutritional quality of these horticultural products is known to be affected by the growing conditions. So, the knowledge about the influence of a hydroponic system production on the nutritional profile of arugula sprouts and microgreens should be considered to optimize their nutritional quality. Nevertheless, a standardized cultivation method for arugula sprouts and microgreens and the nutritional comparison between both have not been previously reported. Therefore, this study aimed to fill this knowledge gap and provide valuable insights into the nutritional quality, evaluating the phytochemical profile of arugula sprouts and microgreens grown organically in a hydroponic system.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Growing conditions

Arugula (*Eruca sativa* L.) seeds were purchased from Isla Sementes Ltda. (Rio Grande do Sul, Brazil). The cultivation was carried out at the Laboratory of Bioactive Compounds of the Institute of Food Science and Technology of the Federal University of Rio Grande do Sul (ICTA/UFRGS), according to the methodology proposed by Di Gioia et al. (2017), with some modifications:

Seeds were placed in phenolic foams with central holes and soaked in water for 48 h to promote germination according to the supplier's recommendation. Plastic trays were prepared by placing these mats of phenolic foam into each tray to create a floating hydroponic system. Sown trays were placed on top of perforated polystyrene boards and irrigated using a nutrient solution prepared with potable water containing (mg L^{-1}): 6% nitrogen, 9% phosphorus, 29% potassium, 2.7% magnesium, 5% sulfur, 0.2% iron, 0.05% manganese, 0.02% zinc, 0.05% boron, 0.03% copper, 0.002% cobalt, 0.006% nickel, and 0.01% molybdenum. It was used an air pump to oxygenate the nutrient solution. Seeded trays were kept at 25°C in a controlled environment and were exposed to sunlight for a 12 h photoperiod.

Sprouts were harvested after 7 days of germination, while the microgreens were

harvested after 14 days. Samples were freeze-dried and stored at -80 °C until further analyses. Three replicates were taken every harvesting time.

In this work, the cultivation methods such as nutrient solution, photoperiod, and the material used for germination were standardized for arugula sprouts and microgreens, allowing a more reliable comparison of the results found.

2.2 Methods

2.2.1 Proximate composition

The proximate analysis of the samples was carried out as follows AOAC (1995): ashes were determined by muffle furnace; proteins content (N × 6.25) was determined by Kjeldahl nitrogen; lipid content was determined by Bligh Dyer method; the enzymatic-gravimetric procedure determined fiber content and total carbohydrates were calculated by the difference of protein, lipid, and ash. The caloric value was determined from a portion of 100 grams of the sample. All analyses were performed in triplicate, and the results were expressed on dry basis.

2.2.2 Ascorbic acid

Ascorbic acid content was determined according to Rizvi et al. (2023), with slight modifications. 1g of each sample was macerated with 20 mL of 0.05 M sulfuric acid 96% and vortexed for 1 min. The extracts were centrifuged at 25400g for 15 min. The supernatants were filtered, and the ascorbic acid was detected with an Agilent 1200 series HPLC system (Agilent, Santa Clara, CA) at 254nm. Chromatographic conditions: C18 column; isocratic mobile phase (100 mM phosphate buffer, pH = 3.0); 0.6 mL/min rate. The results were expressed on dry basis.

2.2.3 Antioxidant capacity

The 2,2′-azinobis-(3-ethylbenzothiozoline-6-sulphonic acid) ($ABTS^{\bullet}$) scavenging activity was determined using the protocol of Re et al. (1999). Absorbance was measured using a spectrophotometer (Shimadzu UV-1800) at 734 nm after 6 min. Results were

expressed as Trolox Equivalent Antioxidant Capacity ($TEAC_{ABTS}$) in milligrams Trolox Equivalent (TE) per gram of fresh weight.

2.2.4 Carotenoids

Carotenoids were evaluated according to Rodrigues et al. (2013). These compounds were extracted using 80% acetone, centrifuged (5 min, 349×*g*), and filtrated through a 0.45-μm nylon membrane syringe filter (VWR International, USA). Chromatographic conditions: HPLC 10A system (Shimadzu, Japan) equipped with a diode array (SPD-M 10A VP) detector; YMC carotenoid column (YMC, Japan). The results were expressed on dry basis. Peaks were detected at 450 nm, and the identification and quantification were done by comparing the sample peaks' retention times and their respective standard (Table Supplementary (TS) 1).

2.2.5 Vitamin A

Vitamin A activity was calculated according to Rizvi et al. (2023) based on retinol equivalent activity ($\mu g.RAE^{-1}$). The following carotenoids were considered in this study: 12 μg β-carotene = 1 equivalent of retinol activity, 24 μg α-carotene = 1 equivalent of retinol activity, and 24 μg β-cryptoxanthin = 1 equivalent of retinol activity.

2.2.6 Phenolics compounds

2.2.6.1 Folin–Ciocalteu method

Total phenolic content (TCP) was determined using the Folin–Ciocalteu method, following Rodrigues et al. (2013). Samples were added to the extraction solvent media in a proportion of 1:120 (w/v). The extraction solvent comprised 0.35% v/v of formic acid solution in 20% v/v of acetone in distilled water. The slurry was concentrated in a rotary evaporator for acetone removal. The extraction was repeated five times, even without extract color. The absorbance was measured at 765 nm and conducted in triplicate. Gallic acid was used as the standard for a calibration curve, and the results were expressed as gallic acid equivalents.

2.2.6.2 HPLC-DAD-MS/MS method

The extraction of the phenolic compounds followed the methodology described by Rodrigues et al. (2013). Exhaustive extraction of the phenolic compounds was carried out. This procedure was performed in triplicate and repeated five times until the absence of color. The identification of phenolic compounds was performed in chromatography HPLC system (Shimadzu, Japan) connected to an array diode detector (Shimadzu SPD-M20A) and a mass spectrometer (MS) (Bruker Daltonics, Germany). A C18 Phenomenex SynergiTM column (Allcrom, Brazil) was used.

The spectra were obtained between 200 and 600 nm, and the chromatograms were processed at 280, 320, 360 nm, and 520 nm. The phenolic compounds were identified based on the order of elution and retention time on the column, characteristics of the UV-VIS and MS spectra compared to standards analyzed under the same conditions (Table Supplementary (TS) 1) and data available in the literature. In addition, the compounds were quantified by HPLC-DAD-MS/MS using an analytical curve, as shown in (Figure Supplementary (FS) 1).

2.2.7 Chlorophylls

0.4g of each sample were macerated with 10% (w/v) of 80% (v/v) acetone–water and then vortexed for 10 min. The homogenized tissue was centrifuged (5000g for 15 min), and the supernatants were filtered. Samples were analyzed in UV–VIS spectrophotometer with the absorbance of the solution mixture at 647 nm for chlorophyll "a" and 663 nm for chlorophyll "b", and the concentration was determined using the CHLs equations, according to Lichtenthaler & Wellburn (1983). The results were expressed on dry basis.

2.3 Statistical analysis

Data were subjected to an analysis of normality and homoscedasticity, and a variance analysis (ANOVA) and Tukey's multiple range test was used to separate the means. Statistical significance was assessed at $p \leq 0.05$, using the software SPSS Statistics (21 version, IBM).

3. RESULTS AND DISCUSSION

3.1 Proximate composition

The content of moisture, ash, protein, carbohydrate, lipid, total, soluble, and insoluble fiber, and caloric value of arugula sprouts and microgreens, are listed in Table 1.

Table 1. Proximate composition of arugula sprouts and microgreens

Parameters (%)	**Samples**	
	Sprouts	Microgreens
Moisture*	93.30±2.81[a]	68.65±0.72[b]
Ash	8.90±0.60[b]	14.52±2.75[a]
Lipid	3.86±0.12[b]	5.58±1.18[a]
Protein	24.84±1.79[a]	18.26±1.97[b]
Carbohydrate	12.22±6.02[b]	30.67±3.43[a]
Total fiber	34.38±2.16[a]	28.78±4.58[a]
Insoluble fiber	19.65±0.87[a]	22.05±4.73[a]
Soluble fiber	11.54±0.06[a]	6.73±0.16[b]
Kcal(/g^{-1})*	14.64±0.22[b]	15.27±0.23[a]

Means followed by the same letter horizontally do not show statistically a significant difference ($p>0.05$). Results are expressed on dry basis. *Results expressed on wet basis.

The results showed that arugula sprouts and microgreens differed statistically in chemical composition and caloric value. The arugula sprout samples showed higher values of moisture (93.30%) and protein (24.84%) compared to the content observed in arugula microgreens (18.26%). The increase in protein content can be attributed to the increased metabolic activity in the initial stages of germination, inducing protein synthesis. The enzymatic hydrolysis of reserve proteins– for generating amino acids and peptides– is used to synthesize new hydrolytic enzymes (Taiz et al., 2015).

Conversely, microgreens samples showed higher amounts of ash, lipids, and carbohydrates, specifically 14.52, 5.58 and 30.67%, respectively. Kowitcharoen et al. (2021) analyzed Brassicaceae family microgreens on wet basis and showed lipid levels

lower than that detected in this paper (0.48% versus 0.84%) and higher protein concentration (2.47% versus 0.94%). The cultivation conditions in the work of these authors were different, which may explain this difference.

According to Vale et al. (2015), red cabbage sprouts, regardless of the presence or absence of light during growth, also obtained a moisture content of around a 93%. Under a 16 h light photoperiod, these vegetables showed similar protein to the arugula sprout samples (26.95±0.46 versus 24.84±1.79) and lower fiber contents (25.04 ± 0.06).

As stated by Zielińska-Dawidziak (2021), the nutritional value of sprouts depends on the species of sprouted plants and the applied conditions. Generally, seeds start their development process with high levels of phytochemicals, which can help them adapt and grow under fluctuating environmental conditions (Oh & Rajashekar, 2009).

In the Drozdowska et al. (2020) studies, red cabbage sprouts (*Brassica oleracea* var. capitata f. rubra) showed similarity to our sprouts in terms of total fiber (30.2 versus 34.3%) and carbohydrate (12.69 versus 12.22 %). According to Erba et al. (2018), during the sprouting period, a decrease in the content of molecules used in metabolism as an energy source was not founded, despite their involvement in the development processes of plants. However, in this period, the hydrolysis of macromolecules was observed, making nutrients more bioavailable.

López-Cervantes et al. (2013) found significant differences in the nutritional composition and the phytochemical level in the different stages of broccoli germination. Distinct sprouts and microgreens also differed in their chemical composition ($p < 0.05$) in Wojdylo et al. (2020) studies. These authors observed that microgreens and sprouts could be especially important for humans to stay healthy and avoid diseases associated with oxidative stress.

With the increase in life expectancy and the current lifestyle, non-communicable chronic diseases such as obesity, cardiovascular disease, cancer, and type 2 diabetes are increasingly common. As such, a plant-rich diet is often recommended as a preventative measure, and in this regard, sprouts and microgreens seem highly desirable products (Wojdylo et al., 2020).

3.2. Carotenoids, pigments, and vitamins

Concerning the identification and quantification of carotenoids, zeaxanthin, and β-carotene were the predominant carotenoids of the total carotenoid content of arugula seeds (Table 2).

Table 2. Bioactive compounds and antioxidant capacity of arugula sprouts and microgreens

Parameters	**Samples**	
	Sprouts	Microgreens
Carotenoids (µg/g)		
Total	74.00±26.60[a]	11.88±0.28[b]
Lutein	4.97±0.72[a]	0.82±0.02[b]
Zeaxanthin	41.84±1.36[a]	1.48±0.08[b]
Cryptoxanthin	6.80±1.01[a]	0.91±0.03[b]
α-carotene	0.65±0.22[a]	0.64±0.22[a]
β-carotene	19.83±15.05[a]	8.12±0.35[b]
Vitamin A (µg.RAE^{-1})	416,76±113,15[a]	134,64±45,63[b]
Chlorophyll (mg.g^{-1})		
Total	4.37±0.03[a]	0.80±0.13[b]
A	1.63±0.04[a]	0.37±0.11[b]
B	2.74±0.06[a]	0.42±0.16[b]
Ascorbic acid (mg.g^{-1})	1.22±0.02[b]	5.17±2.30[a]
Total phenolic content (mg.g^{-1})	1310±0.40[b]	3360±0.31[a]
Total antioxidant capacity (µMol/g)	6.70±0.36[a]	6.80±0.21[a]

Means followed by the same letter horizontally do not show a statistically significant difference ($p>0.05$). Results are expressed on dry basis.

Samples of arugula microgreens showed levels of lutein, zeaxanthin, cryptoxanthin, and β-carotene lower than those observed in arugula sprout samples. The

major differences were observed in zeaxanthin content since a value 28-fold higher was observed in sprout samples regarding microgreens. According to Kopsell et al. (2012), under high light, violaxanthin is transformed into zeaxanthin via antheraxanthin to improve photoprotection since these carotenoids of the xanthophyll cycle have an important role in adapting plants to changing light intensities.

Lutein in sprout samples registered a value of 4.9 $\mu g.g^{-1}$ fw, 6.1-fold higher than arugula microgreens. Similarly, cryptoxanthin in sprouts noted a value 7.0-fold higher than in microgreens samples. The concentrations of α-carotene were similar in both samples. Total chlorophyll, chlorophyll a, and chlorophyll b exhibited significant differences between cultivars. At the microgreens stage, total chlorophyll content was 4.0-fold lower than the sprouts stage. Previous studies showed similar pigment content in Brassicaceae microgreens (Zhang et al., 2021). It is expected to find lower concentrations of chlorophylls and carotenoids, considering that microgreens are mainly composed of cotyledons and only partially developed true leaves (Bulgari et al., 2016).

The chlorophyll content of microgreens in this study were statistically different from sprouts, but similar to those found by Fukalova et al. (2021), when analyzing the phytochemical profile of the same family, but with microgreens of *Diplotaxis erucoides* L., a weed species from southern England. The authors detected means of 0.92±0.20; 0.33±0.05, and 1.25±0.25, respectively. Similar results were also found for arugula microgreens in the study by Bulgari et al. (2016): 0.74±0.02; 0.26±0.01 and 1.00±0.03, respectively.

Lester et al. (2010) found higher levels of ascorbic acid and carotenoids in spinach sprouts than in mature leaves of the same vegetable. In this paper, microgreens showed 4.2-fold more vitamin C concentration than sprouts. On the other hand, sprouts showed 3.0-fold more retinol activity than microgreens. According to Ross & Moran (2020), an explanation for interindividual variability in responses to provitamin A carotenoids is due to carotenoid cleavage genes that affect the bioconversion of provitamin A carotenoids to vitamin A. In the case of ß-carotene, cleavage in the small intestine is a major source of vitamin A, especially for people whose diets are mainly plant-based.

3.3. Phenolic compounds and total antioxidant capacity

According to Xiao et al. (2019), microgreens from the Brassicaceae family are considered good sources of carotenoids, glucosinolates, and total phenolic compounds. Regarding this last parameter, the arugula microgreens samples presented 2.6 times more levels detected in the sprouts (Table 3).

Table 3. Chromatographic and Spectroscopic Characteristics of phenolic compounds in arugula sprouts and microgreens obtained by LC-DAD-ESI-MS/MS

Peak	Compound	T_R (min)[a]	λ_{max} (nm)[b]	$[M-H]^-$ (*m/z*)	MS^2 (-) (*m/z*)
		Sprouts			
38	Luteolin 7-O-diglucuronide	22.8 – 23.6	328	638.1119	638,11
		Microgreens			
32	Apigenin 7-O-glucoside or 6-C-glucoside	20.6 – 21.6	269	432.1056	433,18
33	Isorhamnetin 3-O-glucoside 7-O-rhamnoside	21.8 -23.1	266	624.1690	625,10
34	Diosmin or Neodiosmin	21.9 – 23.2	337	608.1741	609,10
35	Gallic acid 3-O-gallate	22.7 – 23.8	328	322.0324	323,09
	Myricetin 3-O-galactoside or 3-O-glucoside			480.0903	481,07
	Luteolin 7-O-diglucuronide			638.1119	638,11
43	Quercetin 3-O-(6"-malonyl-glucoside)	29.9 - 31.0	327	550.0009	551,09

[a] Retention time on the C18 LiChrospher® (5 μm) column. [b] Maximum absorbance. Solvent: gradient of 0.5% formic acid in water and acetonitrile with 0.5% formic acid.

Oh et al. (2010) also observed higher concentrations of total phenolics and total antioxidant capacity after 7 days of germination compared to mature lettuce leaves. Oh & Rajashekar (2009), when studying broccoli and radish sprouts, suggest that the phytochemical change composition depends on the plant's age.

In this study, arugula sprouts and microgreens did not differ statistically regarding antioxidant capacity and showed a mean content of around 6.75 µMol/gdw. Ghoora et al. (2020) found higher values for mustard and radish microgreens (10.9 and 15.4 µMol, respectively), with statistical differences among species. Broccoli and radish sprouts, in the work of Wojdylo et al. (2020), both showed the antioxidant capacity of 1.8 µMol, while the kale and radish microgreens showed a content of 1.0 and 0.6 µMol, respectively, with a statistical difference.

Secondary metabolism, such as polyphenols, is produced through the phenylpropanoid pathway. This metabolic pathway makes tissues resistant to many stresses that may occur later in postharvest handling, storage, and distribution, as well as can suffer the influence of preharvest determinants, such as light intensity, temperature variations, and nutrient composition (Martinez et al., 2021).

A key gateway enzyme in the phenylpropanoid pathway is phenylalanine ammonia-lyase (PAL). In response to environmental stresses, an increase in PAL activity and activation of several genes have been shown, leading to the biosynthesis of a wide array of phenolics and flavonoids. For example, the antioxidant capacity and the total phenolic content of lettuce plants increased significantly in response to all the environmental shocks, such as heat, chilling, and highlight (Oh et al., 2009).

With regard to cultivation conditions, the sowing rate, the use of fertilizers, and the dose of light received should be considered (Choe et al., 2018). LED light, for example, can promote the accumulation of phenolic compounds and provide greater antioxidant capacity for species grown under this system (Zhang et al., 2020). As the time between sowing and harvesting microgreens differs between species, growers should select crops with a similar growth rate to harvest the crop all at once (Kyriacou et al., 2018).

This study's total phenolic content in arugula microgreen samples was 3360 $mg.g^{-1}$±0.31. It is about 80-fold higher than the TPC in many species of mature vegetables, including radish, cabbage, and cauliflower, and 20-fold more elevated than the TPC in broccoli (Song et al., 2010).

3.4 The profile of extractable phenolic compounds

In order to explore the beneficial effects of phenolic compounds on human health, the profile and quantification of specific phenolic compounds in arugula sprouts and microgreens were assessed (Tables 3 and 4). To the best of our knowledge, this is the first report of quantification and comparison of phenolic compounds between arugula sprouts and microgreens.

Table 4. Phenolic compounds quantification (mg/L) of arugula sprouts and microgreens

λ (nm)	Compound	Samples	
		Sprouts	Microgreens
280	Galic acid	23.12±4.36[a]	7.54±2.56[b]
	Catechin	23.00±2.40[a]	9.74±2.28[b]
	Hidroxibenzoic acid	29.33±4.81[b]	55.10±4.23[a]
	Epicatechin	102.66±5.64[b]	161.62±14.24[a]
	Vanillic acid	7.63±1.18[a]	4.67±00.42[b]
	Epicatechin gallate	100.87±12.15[a]	78.49±29.21[a]
	p-Cumaric acid	1.24±0.35[a]	1.19±0.09[a]
320	Chlorogenic acid	2.91±1.92[b]	5.93±0.95[a]
	Caffeic acid	14.43±2.69[b]	25.79±2.26[a]
	Ferulic acid	6.45±0.93[a]	6.40±0.60[a]
360	Rutin	12.62±2.11[a]	11.24±0.88[a]

Values are the mean of 3 replications (± standard deviation). Different uppercase superscript letters in the same line indicate a statistically significant differences between treatments for the same compound by the Tukey test ($p<0.05$).

The profile of extractable phenolic compounds of the analyzed samples was mainly characterized by the presence of derivatives of polyphenols and phenolic acids (Table 3), found mainly as O-glycosides. This was the case of the compounds Luteolin 7-O-diglucuronide in sprouts and microgreens, Apigenin 7-O-glucoside or 6-C-glucoside, Isorhamnetin 3-O-glucoside 7-O-rhamnoside, Gallic acid 3-O-gallate,

Myricetin 3-O-galactoside or 3-O-glucoside and Quercetin 3-O-(6''-malonyl-glucoside) in microgreens. Arugula microgreens samples showed a higher amount and variety of flavonol glycosides compared to arugula sprout samples. According to Cartea et al. (2011), these compounds are present in high concentrations in the epidermis of leaves.

Flavonols, such as catechin and epicatechin, have different mechanisms of action depending on the concentration they reach in the target tissue. Micromolar levels would be sufficient to eliminate free radicals and metals that induce oxidative stress (Grzesik et al., 2018). Information about the conjugation of sugar to the polyphenolic section may provide additional knowledge on their nutritional importance since the sugar moiety is an important determinant of the bioavailability of flavonols (Vrhovsek et al., 2012).

Quercetin, myricetin, kaempferol, and isorhamnetin are the most abundant flavonols in plant-based foods (Cartea et al., 2011) and were present in microgreens samples in this paper. Kyriacou et al. (2018) analyzed polyphenols from thirteen different microgreens, eight of which belong to the Brassicaceae family, and verified the majority presence of kaempferol glycosides, quercetin, and isorhamnetin. According to the authors, the polyphenol composition profiles differed significantly between microgreen species.

The quantification of these compounds (Table 4) by internal standardization using the curves of 12 analytical standards of phenolic compounds (FS 1). Catechin showed a statistically significant difference between sprouts (23 mL/L) and microgreens (9.7 mL/L). This compound is an effective antioxidant and might be a mediator in cardiovascular health (Grzesik et al., 2018).

At the wavelength of 320nm, the amount of chlorogenic and caffeic acid was higher in microgreens, with a statistically significant difference. Pajak et al. (2014) studies showed that among free phenolic acids, caffeic and gallic acid occurred in the largest amounts in broccoli sprouts (4.12 mg for both acids). In this paper, gallic acid was 23.12 mg for arugula sprouts and 7.54 mg for arugula microgreens. The amounts of the flavonoids presented in their work were inconsiderable, except for radish sprouts, because of the quercetin (8.94 mg) predominance. The values for arugula in this study were 2.91 mg for sprouts and 5.93 mg for microgreens.

In the Kyriacou et al. (2019) analysis of polyphenols, they have revealed substantial genotypic differences. As reported by these authors, the profile and concentration of phenolics in plants during ontogeny depend on their genetic constitution, even with cultivars of the same species.

4. CONCLUSION

This study followed specific cultivation conditions in order to elucidate several questions. In the search for new functional food sources, this study improved our knowledge of the nutritional profile of arugula sprouts and microgreens, revealing that they are good sources of proteins, fibers, carotenoids, and phenolic compounds. Therefore, the production, both on a commercial and domestic scale and consumption of these vegetables should be used daily due to their health-promoting properties. It is suggested that further investigations be carried out on the impact of different cultivation methods and specific conditions on microgreens and sprouts to promote the highest levels of desired nutrients and bioactive compounds.

Acknowledgment

Capes for the study fellowship.

Ethical Guidelines

Ethics approval was not required for this research.

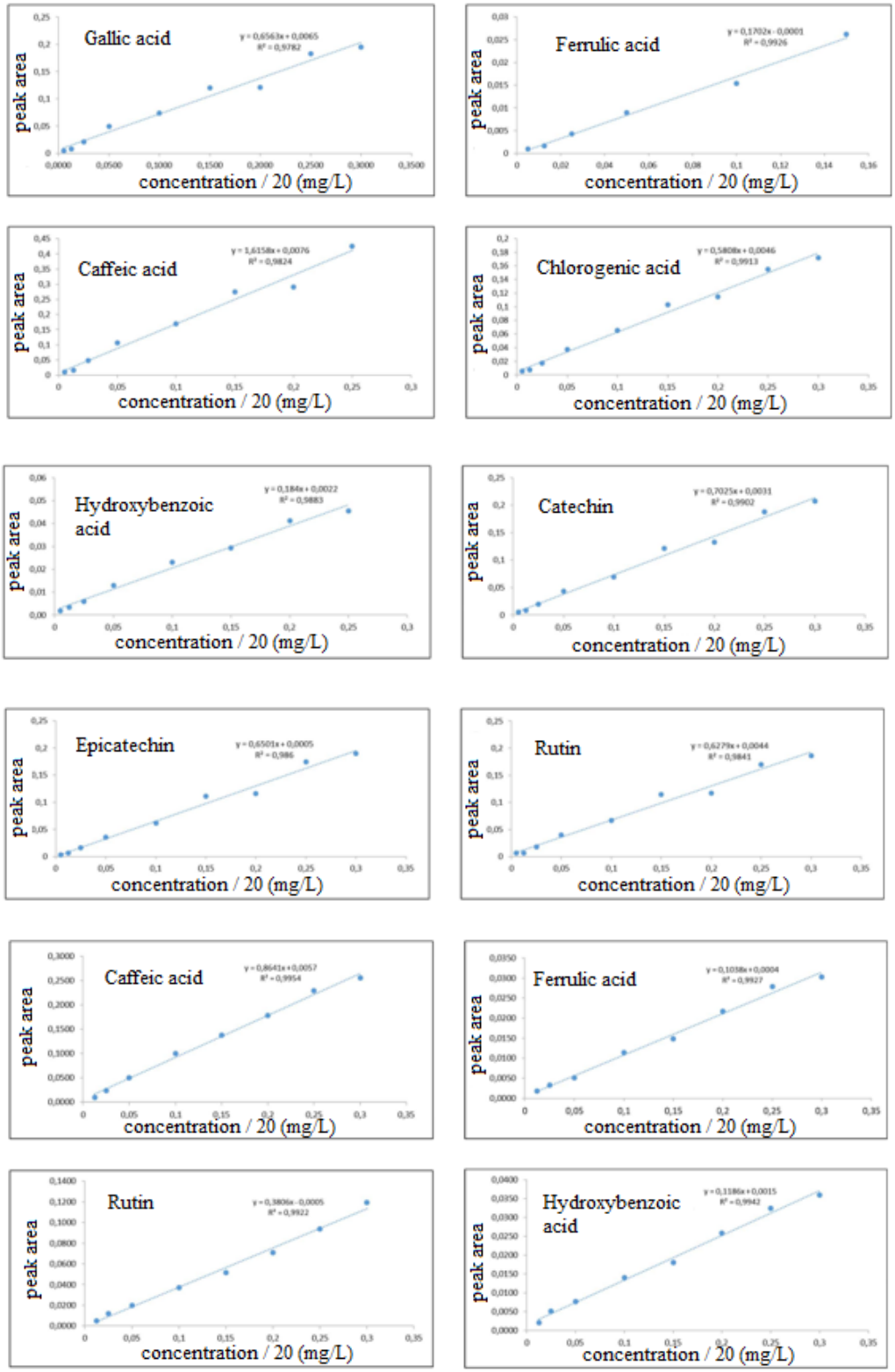
Data Availability

Data sharing is not applicable to this article.

Table Supplementary 1. Work range, determination coefficients (r^2), the limit of quantification (LOQ), the limit of detection (LOD) of carotenoids and phenolics compounds

Standards	**Work range**	**r^2**	**LOD**	**LOQ**
Lutein ($\mu g.g^{-1}$)	1-65	0.9991	6.90×10^{-3}	1.15×10^{-2}
Zeaxanthin ($\mu g.g^{-1}$)	1- 40	0.9997	9.56×10^{-2}	1.59×10^{-2}
Cryptoxanthin ($\mu g.g^{-1}$)	3 -100	0.9911	2.11×10^{-2}	3.51×10^{-2}
α-carotene ($\mu g.g^{-1}$)	2 - 25	0.9934	1.97×10^{-2}	3.28×10^{-2}
β-carotene ($\mu g.g^{-1}$)	5 - 50	0.9998	6.53×10^{-2}	10.89×10^{-2}
Lycopene ($\mu g.g^{-1}$)	10 - 100	0.9977	7.0×10^{-3}	3.3×10^{-2}
Caffeic acid	0.36 - 12	0.9961	0.97	2.94
Chlorogenic acid	0.75 - 24	0.9960	0.77	2.34
Coumaric acid	0.05 - 12	0.9935	0.28	0.86
Ellagic acid	0.05 - 12	0.9925	0.55	1.67
Ferulic acid	0.05 - 12	0.9940	0.85	2.56
Gallic acid	0.75 - 24	0.9963	0.62	1.87
Catechin	0.70 - 70	0.9905	1.50	4.53
Epigallocatechin gallate	0.10 - 70	0.9964	0.09	0.26
Kaempferol	0.05 - 12	0.9925	0.03	0.11
Luteolin	0.10 - 50	0.9931	0.34	1.04
Myricetin	0.28 - 50	0.9938	1.23	3.71
Quercetin	0.40 - 50	0.9942	0.71	2.15
Quercetin 3-glucoside	0.11 - 50	0.9997	0.07	0.20
Rutin	0.11 - 15	0.9931	0.18	0.54

Figure Supplementary 1. Curves of the analytical standards of phenolic compounds



REFERENCES

AOAC. *Official Methods of Analysis of AOAC International*, 16th ed.; AOAC International: Rockville, MD, USA, 1995; Volume 1.

Bulgari, R., Baldi, A., Ferrante, A., Lenzi, A. (2016). Yield and quality of basil, Swiss chard, and rocket microgreens grown in a hydroponic system. *New Zealand Journal of Crop and Horticultural Science*, **45**(2): 119–129.

Cartea, M., Francisco, M., Soengas, P. (2011). Phenolic compounds in Brassica vegetables. *Molecules*, **16**.

Choe, U., Yu, L., Wang, T. (2018). The science behind microgreens as an exciting new food for the 21th century. *Journal of Agricultural and Food Chemistry*, **66**(44): 11519-11530.

Di Gioia, F., Renna, M., Santamaria, P. Sprouts, microgreens and "Baby Leaf" vegetables. In *Minimally Processed Refrigerated Fruits and Vegetables*; Yildiz, F., Wiley, R.C., Eds.; Springer: Boston, MA, USA, 2017; pp. 403–432.

Drozdowska, M., Leszczynska, T., Koronowicz, A. (2020). Young shoots of red cabbage are a better source of selected nutrients and glucosinolates in comparison to the vegetable at full maturity. *European Food Research and Technology*, **246**: 2505-2515.

Erba, D., Angelino, D., Marti, A., Manini, F., Faoro, F., Morreale, F., Pellegrini, N., Casiraghi, M. (2019). Effect of sprouting on nutritional quality of pulses. *International Journal of Food Science and Nutrition*, **70**: 30–40.

Fukalova, T., García Martínez, M., Raigón, M. (2021). Five undervalued edible species inherent to autumn-winter season: nutritional composition, bioactive constituents and volatiles profile. *PeerJ*, **9**:e12488.

Ghoora, M., Haldipur, A., Srividya, N. (2020). Comparative evaluation of phytochemical content, antioxidant capacities and overall antioxidant potential of select culinary microgreens. *Journal of Agriculture and Food Research*, **2**:1-7.

Ghoora, M., Babu, D., Srividya, N. (2020a). Nutrient composition, oxalate content and nutritional ranking of ten culinary microgreens. *Journal of Food Composition and Analysis*, **91**:1-10.

Grzesik, M., Naparlo, K., Bartosz, G., Sadowska-Bartosz, I. (2018). Antioxidant properties of catechins: Comparison with other antioxidants. *Food Chemistry*, **241**: 480–492.

Johnson, S., Prenni, J., Heuberger, A., Isweiri, H., Chaparro, J., Newman, S., Uchanski, M.; Omerigic, H., Michell, K., Bunning, M. (2021). Comprehensive Evaluation of Metabolites and Minerals in 6 Microgreen Species and the Influence of Maturity. *Current Developments in Nutrition*, **5**: nzaa180.

Katsenios, N., Christopoulos, M., Kakabouki, I., Vlachakis, D., Kavvadias, V. Efthimiadou, A. (2021). Effect of Pulsed Electromagnetic Field on Growth, Physiology and Postharvest Quality of Kale (*Brassica oleracea*), Wheat (*Triticum durum*) and Spinach (*Spinacia oleracea*) Microgreens. *Agronomy*, **11**(7):1364.

Kowitcharoen, L., Phornvillay, S., Lekkham, P., Pongprasert, N., Srilaong, V. (2021). Bioactive Composition and Nutritional Profile of Microgreens Cultivated in Thailand. *Applied Sciences*, **11**(17):7981.

Kopsell, D., Pantanizopoulos, N., Sams, C., Kopsell, D. (2012). Shoot tissue pigment levels increase in 'Florida Broadleaf' mustard (Brassica juncea L.) microgreens following high light treatment. *Scientia Horticulturae*, **140**: 96-99.

Kyriacou, M., De Pascale, S., Kyratzis, A., Rouphael, Y. (2017). Microgreens as a Component of Space Life Support Systems: A Cornucopia of Functional Food. Frontiers. *Plant Science*, **8**(1587).

Kyriacou, M., El-Nakhel, C., Graziani, G., Pannico, A., Soteriou, G., Giordano, M., Rouphael, Y. (2018). Functional quality in novel food sources: genotypic variation in the nutritive and phytochemical composition of thirteen microgreens species. *Food Chemistry*, **277**:107 – 118.

Kyriacou, M., El-Nakhel, C., Pannico, A., Graziani, G., Soteriou, G., Giordano, M., Zarrelli, A., Ritieni, A., De Pascale, S. Rouphael, Y. (2019). Genotype-Specific Modulatory Effects of Select Spectral Bandwidths on the Nutritive and Phytochemical Composition of Microgreens. *Frontiers in Plant Science*, **10**:1501.

Lichtenthaler, H. K., Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, **11**: 591-592.

Lester, G., Hallman, G., Pérez, J. (2010). A gamma-Irradiation dose: effects on baby-leaf spinach ascorbic acid, carotenoids, folate, alpha-tocopherol, and phylloquinone concentrations. *Journal of Agricultural and Food Chemistry*, **58**(8): 4901-6.

López-Cervantes, J., Tirado-Noriega, L. G., Sánchez-Machado, D. I., Campas-Baypoli, O. N., Cantú-Soto, E. U., & Núñez-Gastélum, J. A. (2013). Biochemical composition of broccoli seeds and sprouts at different stages of seedling development. *International Journal of Food Science & Technology*, **48**(11), 2267-2275.

Martínez, C., Valenzuela, J., Jamilena, M. (2021). Genetic and Pre- and Postharvest Factors Influencing the Content of Antioxidants in Cucurbit Crops. *Antioxidants*, **10**(6): 894.

Oh, M., Rajashekar, C. (2009). Antioxidant content of edible sprouts: Effects of environmental shocks. *Journal of the Science of Food and Agriculture*, **89**(13): 2221-2227.

Oh, M., Trick, H., Rajashekar, C. B. (2009). Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce. *Journal of plant physiology*, **166**(2): 180-191.

Pająk, P., Socha, R., Gałkowska, D., Rożnowski, J., Fortuna, T. (2014). Phenolic profile and antioxidant activity in selected seeds and sprouts. *Food Chemistry*, **143**: 300-306.

Re, R, Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, **26**: 1231-1237.

Rizvi, A., Sharma, M., Saxena, S. (2023). Microgreens: A Next Generation Nutraceutical for Multiple Disease Management and Health Promotion. *Genetic Resources and Crop Evolution*, **70**: 311-332.

Rodrigues, E., Mariutti, L. R. B., Mercadante, A. Z. (2013). Carotenoids and Phenolic Compounds from Solanum sessilif lorum, an Unexploited Amazonian Fruit, and Their Scavenging Capacities against Reactive Oxygen and Nitrogen Species. *Journal of Agricultural and Food Chemistry*, **61**(12): 3022–3029.

Ross, C., Moran, N. (2020). Our Current Dietary Reference Intakes for Vitamin A—Now 20 Years Old, *Current Developments in Nutrition*, 4(10): nzaa096.

Shah, S., Houborg, R., Mccabe, M. (2017). Response of chlorophyll, carotenoid and SPAD-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.) *Agronomy*, **7**: 61.

Song, W., Derito, C., Liu, M., He, X., Dong, M., Liu, R. (2010). Cellular antioxidant activity of common vegetables. *Journal of Agricultural and Food Chemistry*, **58**: 6621–6629.

Taiz, L., Zeiger, E., Møller, I. M., Murphy, A. (2015). *Plant Physiology and Development* (No. Ed. 6). Sinauer Associates Incorporated.

Vale, A., Santos, J., Brito, V., Peixoto, V., Carvalho, R., Rosa, Oliveira, M. (2015). Light influence in the nutritional composition of *Brassica oleracea* sprouts. *Food Chemistry*, **178**: 292-300.

Vrhovsek, U., Masuero, D., Palmieri, L., Mattivi, F. (2012). Identification and quantification of flavonol glycosides in cultivated blueberry cultivars. *Journal of Food Composition and Analysis*, **25**(1): 9–16.

Wojdyło A, Nowicka P, Tkacz K, Turkiewicz IP. (2020). Sprouts vs. Microgreens as Novel Functional Foods: Variation of Nutritional and Phytochemical Profiles and Their In vitro Bioactive Properties. *Molecules*, **25**(20): 4648.

Xiao, Z., Lester, G., Luo, Y. (2012). Assessment of vitamin and carotenoid concentrations of emerging food products: edible microgreens. *Journal of Agricultural and Food Chemistry,* **60**: 7644-7651.

Xiao, Z., Rausch, S., Luo, Y., Sun, J., Yu, L., Wang, Q., Chein, P., Stommel, J. (2019). Microgreens of Brassicaceae: Genetic diversity of phytochemical concentrations and antioxidant capacity. *LWT,* **101**: 731-737.

Zhang, X., Bian, Z., Yuan, X., Chen, X., Lu, C. (2020). A review on the effects of light-emitting diode (LED) light on the nutrients of sprouts and microgreens. *Trends in Food Science & Technology*, **99**: 203-216.

Zhang, Y., Xiao, Z., Ager, E., Kong, L., Tan, L. (2021). Nutritional quality and health benefits of microgreens, a crop of modern agriculture. *Journal of Future Foods,* **1**(1): 58-66.

Zielinska-Dawidziak, M. (2021). Influence of stress conditions on the quality of obtained sprouts – modification on their chemical. *Quality Assurance and Safety of Crops & Foods*, **13**(2): 1-12ISSN.

5.3 ARTIGO 3 – "Qualidade pós-colheita de microgreens de rúcula (*Eruca sativa*) determinada por parâmetros microbiológico, físico-químico e sensorial"

foods

Order Article Reprints

Open Access Article

Postharvest Quality of Arugula (*Eruca sativa*) Microgreens Determined by Microbiological, Physico-Chemical, and Sensory Parameters

by **Marina R. Komeroski** [1,*], **Thais Beninca** [1], **Keyla A. Portal** [2], **Patrícia S. Malheiros** [1], **Tâmmila V. Klug** [3], **Simone H. Flores** [1] and **Alessandro O. Rios** [1]

[1] Postgraduate Program in Food Science, Institute of Food Science and Technology, Federal University of Rio Grande do Sul, Porto Alegre 90010-150, Brazil

[2] Department of Nutrition, School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre 90010-150, Brazil

[3] Postgraduate Program in Science and Food Technology, Department of Food Science, Farroupilha Federal Institute, Santa Maria 97050-685, Brazil

[*] Author to whom correspondence should be addressed.

Foods **2024**, *13*(19), 3020; **https://doi.org/10.3390/foods13193020**

Submission received: 31 August 2024 / Revised: 20 September 2024 / Accepted: 21 September 2024 / Published: 24 September 2024

(This article belongs to the Special Issue **Storage and Shelf-Life Assessment of Food Products**)

Abstract: (1) Background: Cultivating microgreens is presenting itself as an excellent market opportunity. Their easy, short, and sustainable production methods are the main reasons they are approved of growers. However, a feature that still prevents its spread is the microbiological risk and its rapid senescence. The present study was conducted to evaluate the post-harvest storage and shelf life of arugula microgreens in different packaging, through microbiological, physico-chemical, and sensory parameters; (2) Methods: These plants were stored at 5 °C in open, vacuum sealed, and under modified atmosphere bags, and tested at 0, 3, 5, 7, and 10 days; (3) Results: Microgreens stored in all packaging were safe for consumption within ten days. Regarding physical and chemical parameters, open packaging proved to be promising, with less weight loss and slower chlorophyll degradation. The sensory analysis demonstrated that the microgreens stored in the vacuum-sealed packaging showed a worsening in quality from the fifth day onwards, for all attributes. However, the MAP presented good scores, with a better visual quality, similar to the fresh microgreens.

Keywords: Brassicaceae microgreens; packaging; shelf-life assessment; microbiological analysis; physico-chemical parameters

1. Introduction

Arugula is one of the most consumed vegetables from the Brassicaceae family in the world [1,2] and the intensity of its aroma, pungency and crunchiness seem to be decisive in consumer acceptance [3]. Microgreens of this vegetable are consumed fresh in salads but are also served as a garnish in other dishes, such as soups and sandwiches [4]. These plants contain significant amounts of important bioactive compounds and minerals [5], often being higher than adult plants of the same species [6], since they receive only light treatments [7] and are preceded by the germination stage [8].

This crop has a fast production cycle [9] and can be produced in greenhouses, in soil or, more commonly, in soilless systems, using solid organic or inorganic growing media or hydroponics [10], which demonstrates the potential of these products to adapt the production of leafy vegetables to different scales [11]. If they are produced hydroponically, the soil is replaced by a substrate and seedlings are fed with a solution containing all the essential elements for their growth [12], and they can be grown organically [13]. Komeroski et al. [14] showed a high 45 protein, total fiber, and soluble fiber content of arugula microgreens grown in this system.

Choosing a culture medium with adequate microbiological characteristics is extremely important to ensure microgreens safe consumption, since the chosen medium may represent a contamination source [15]. For exemple, heat and humidity are the same ideal grow conditions for microgreens and pathogens such as *Salmonella, Listeria*, and *E. coli* O157:H7. These bacteria can infect seeds through small cracks and multiply to high levels during sprouting [16].

Before harvest, the greatest risks of contamination are related to irrigation water, substrate and other factors [15]. In this regard, a delicate balance is required to maintain the lower temperature (0 – 5 °C) and humidity conditions (50 – 85%), that optimizes the quality retention and microgreens shelf life while discouraging the microbes growth and human pathogens [17]. However, data on potential microbiological hazards post-harvest are lacking. Currently, this type of product is packaged for subsequent sale without any disinfectant treatment, increasing consumer health risks.

According to Mir et al. [18], advances in packaging technology will help maintain the quality of microgreens for longer periods and extend their shelf life. In addition to quality parameters, functional information from these plants will help select the specific crop for a particular type of storage.

The modified atmosphere packaging (MAP) extends shelf life with persistent storage temperature and limited oxygen or moisture flows [19] by changing the gas composition, creating an appropriate atmosphere inside the packaging film, and effecting the integrity of the leaf tissue membrane [20]. Nowadays, it is one of the most effective technologies in maintaining the quality and extending the shelf life of fresh produce [21].

This work goal was to evaluate the post-harvest storage and shelf life of arugula microgreens in open, vacuum-sealed and modified atmosphere packaging, through microbiological, sensorial and physico-chemical analysis.

2. Materials and Methods

2.1 Growing conditions

Arugula (*Eruca sativa* L.) seeds were purchased from Isla Sementes Ltda. (Rio Grande do Sul, Brazil). The cultivation was carried out at the Laboratory of Bioactive Compounds of the Institute of Food Science and Technology of the Federal University of Rio Grande do Sul (ICTA/UFRGS), according to the methodology proposed by Di Gioia et al. [7], with some modifications:

Seeds were placed in phenolic foams Green-up® with central holes and soaked in water for 48 h to promote germination according to the supplier's recommendation. The cultivation system used was floating hydroponic. In this case, these sown foams were placed on top of perforated polystyrene boards and irrigated using a nutrient solution prepared with potable water containing: 6% nitrogen, 9% phosphorus, 29% potassium, 2.7% magnesium, 5% sulfur, 0.2% iron, 0.05% manganese, 0.02% zinc, 0.05% boron, 0.03% copper, 0.002% cobalt, 0.006% nickel, and 0.01% molybdenum. An air pump was used to oxygenate the nutrient solution. Seeded trays were kept at 25°C in a controlled environment and were exposed to sunlight for a 12 h photoperiod. Microgreens were harvested after 14 days of germination, cutting the plants manually with scissors a few millimeters above the phenolic foam.

The microgreens were packed (30 g) in polypropylene bags (15 × 20 x 1,2 cm; OTR 50 $cm^3/m^2/24$ h/bar at 23 °C and 75% RH; residual pressure in the vacuum package was <100 Pa; 12 mm of thickness) - TecMaq®, São Paulo - Brazil) in different forms: open packaging (Open), the plastic bag remains with the upper side open; vacuum sealed packaging (Sealed), using the Fastvac® sealer (model TM 250, TecMaq, São Paulo - Brazil) and modified atmosphere packaging (MAP), in the same equipment, with the following gas composition: 5% carbon dioxide, 2.1% oxygen and 92.9% nitrogen, as recommended by Chitarra & Chitarra [20]. After, they were stored at 5 °C in a climate chamber (model NL-41-ALT, New Lab®, São Paulo - Brazil) under a 12-hour photoperiod (light/dark cycle) for until ten days. Sampling was performed after 3, 5, 7 and 10 days of storage. Three packs were sampled at each time and analyzed as independent replicates for shelf-life evaluation.

2.2 Microbiological analysis

2.2.1 *Salmonella spp.* and *Listeria monocytogenes* analysis

The isolation of *Salmonella* spp. and *Listeria monocytogenes* in samples of microgreens stored in open, sealed, and modification atmosphere bags for 0, 5, and 10 days was carried out according to the ISO 6579-1:2017/Amd 1:2020 and ISO 11290-1:2017 methodology, respectively.

For *Salmonella* pre-enrichment, 10 g of the sample was added to 90 mL of buffered peptone water (BPW; HIMEDIA) and incubated at 37 ºC for 24 hours. Subsequently, selective enrichment was carried out in Muller Kauffmann Tetrationate Broth (MKTTn; HIMEDIA) at 37 ºC for 24 hours and Rappaport-Vassilidis Soy Broth (RVS; HIMEDIA) at 37 ºC and 43 ºC for 24 hours. Then, seeding was carried out on Xylose Lysine Desoxycholate Agar (XLD; KASVI) and Hektoen Enteric Agar (HE; HIMEDIA) plates, both being incubated at 37 ºC for 24 hours [21]. Typical colonies were selected and subjected to identification through proteomic analysis using matrix-assisted laser desorption/ionization mass spectrometry and time of flight (MALDI-TOF/MS), model Autoflex Speed (Bruker Corporation, Bremen, Germany), following Barbosa et al. [22].

For pre-enrichment of *Listeria*, 10 g of the sample was added to 90 mL of Half-Fraser broth (HIMEDIA) and incubated at 30 ºC for 25 hours. Subsequently, sowing was carried out on Listeria

Ottaviani & Agosti (ALOA) and OXFORD agar plates, incubating at 37 ºC for 24 hours to 48 hours. Furthermore, 100 μL of the contents of the pre-enrichment tube were transferred to 10 mL of Fraser broth and incubated at 37 ºC for 24 hours. Afterward, seeding was carried out on ALOA and OXFORD plates incubated at 37 ºC for 24 to 48 hours [21]. All experiments were performed in duplicate.

2.2.2 Total Enterobacteriaceae and *Escherichia coli* count

Total Enterobacteriaceae and *Escherichia coli* in samples of microgreens stored in open, sealed packaging and under a modified atmosphere for 0 and 10 days was carried out according to the Petrifilm TM method [23]. Subsequently, the plates were incubated at 35 ºC for 24 hours to count total Enterobacteriaceae and 48 hours to count *Escherichia coli* [21]. Experiments were performed in duplicate.

2.2.3 Mesophilic, psychrophilic, and molds and yeasts count

The total count of mesophilic, psychrophilic, and molds and yeasts in samples of microgreens stored in open, sealed packages and under a modified atmosphere for 0, 5, and 10 days was carried out according to the plating methods APHA 08:2015, APHA 13.61:2015 and APHA 21:2015, respectively. The following media and incubation conditions were used: Counting Standard Agar (PCA) for mesophilic (35 ºC for 48 hours) and psychrotrophilic (7 ºC for 10 days) and Dichloran Rose Bengal Chloramphenicol (DRBC) agar plates for molds and yeasts (25 ºC for 5 days). The results were expressed as log CFU/g [21]. Experiments were performed in duplicate.

2.3 Physicochemical analysis

2.3.1 Color

Microgreen samples were measured using a colorimeter from Konica Minolta® - Osaka/Japan (model Chrona Meter CR400). This equipment identifies the color spectrum in a three-dimensional system (CIE standard *illuminant* D65 and standard *observer* 10), with the vertical axis, "L", referring to the color of the sample from black to white; axis "a", from green to red; and axis "b" from blue to yellow. The L-axis ranges from 0 to 100, with values over 50 being the lighter samples and below 50, the darker ones. All readings were performed in triplicate.

2.3.2 pH, soluble solid content and titratable acidity

Microgreens were ground in a tissue homogenizer Ultra Turrax® (model IKA T25) in a ratio of 1:5 (leaves:water), filtered, and its pH was measured at 25 ° C using a Satra® pH meter (model pHS-3E). The Brix° were measured using an Atago® refractometer (model PAL-3), according to the AOAC methodology [23]. The titratable acidity (TA) was measured by titrating a 10 mL aliquot of microgreen juice extracts with 0.1 mol/L NaOH to the end point of pH 8.2. The results were expressed as the concentrations of $[H^+]$ in the unit of mol/L based on the fresh weight mass of the sample [24].

2.3.3 Weight loss

Weight loss was performed by scaling the microgreens before and after storage. Weight loss was computed according to the following formula [25]:

$$\text{Weight loss (\%)} = (m_0 - m_1)/m_0 \times 100\%$$

where:

m_0 = the weight (g) of microgreens before storage

m_1 = the weight (g) of microgreens after storage

2.3.4 Chlorophylls

The samples (0.4 g) were macerated with 10% (w/v) of 80% (v/v) acetone–water and then vortexed for 10 min. The homogenized tissue was centrifuged (5000 g for 15 min), and the supernatants were filtered. Samples were analyzed in UV–VIS spectrophotometer with the absorbance of the solution mixture at 647 nm for chlorophyll "a" and 663 nm for chlorophyll "b", and the concentration was determined using the CHLs equations, according to Lichtenthaler & Wellburn [26]. The results were expressed on a dry basis.

2.4 Sensory evaluation

Fresh (control, harvested on each day of analysis) and stored microgreens (3, 5, and 7 days) were submitted to sensory analysis at the Laboratory of Sensory Evaluation of the Food Science and Technology Institute (ICTA) of UFRGS, evaluating the following attributes: appearance, texture, taste, odor, color and overall acceptance.

Adult judges (40) of both genders (the average age was ±34 years, ranging from 18–49 and 72.5% of the judges (n = 29) were female, while 27.5% (n = 11) were male) were randomly and

voluntarily recruited to receive a slice of approximately 1g of each formulation, with three-digit random codes, a glass of water to clean the taste buds, and an evaluation sheet for sensory analysis, which contained a hedonic scale of nine points (1 = Dislike extremely, 9 = Like extremely). The analysis was carried out in 20 minutes and the judges signed the Term of Informed Consent in accordance with the rules of research involving human beings.

This procedure was approved by the ethics and research committee of the UFRGS. Number of the Certificate of Presentation of Ethical Review: 65303222.5.0000.5347.

To calculate the acceptability index (AI) of each treatment (>70%= well accept), the following expression was used, as described by Viana [27]:

$$AI\ (\%) = A \times 100 / B \quad (1)$$

where:
A = average grade obtained for each treatment
B = maximum grade given for each treatment

2.5 Statistical analysis

Data regarding shelf life in different packaging were subjected to two-way analysis of variance ANOVA. In all of the data, the mean values were compared using Tukey's test and separated by a least significant difference test ($P < 0.05$). Statistical analysis was performed using the software Statistica 14.0.1.

3. Results and Discussion

Microbiological analyses were performed to evaluate the contamination of the growing media and microbial growth levels on microgreens stored in different packaging. *Salmonella* spp. and *Listeria* monocytogenes were not observed in arugula microgreens, regardless of the day of storage and packaging used (Table 1). The same was observed by Priti et al. [28], who worked with mung bean (*Vigna radiata* L.), lentil (*Lens culinaris* subsp. culinaris), and Indian mustard (*Brassica juncea* L.) microgreens and not detect *Salmonella* and *Listeria* in any of the samples tested.

Table 1. Presence or absence of *Salmonella* spp. and *Listeria* monocytogenes in arugula microgreens stored in different packages for 0, 5 and 10 days.

Samples/time	0 days	5 days	10 days
***Salmonella* spp.**			
Open	Absent	Absent	Absent
Sealed	Absent	Absent	Absent
MAP	Absent	Absent	Absent

***Listeria* monocytogenes**			
Open	Absent	Absent	Absent
Sealed	Absent	Absent	Absent
MAP	Absent	Absent	Absent

Open: open packaging, the plastic bag remains with the upper side open; Sealed: vacuum sealed packaging; MAP: modified atmosphere packaging.

Generally, mesophilic and psychrotrophic bacteria counts and enumeration of total Enterobacteriaceae are useful for indicating the shelf-life duration and microbial quality of foods [31]. Regarding the count of total Enterobacteriaceae (Table 2), the presence of these microorganisms was verified, although in accordance with the resolution (RDC 724/2022) and normative instruction (IN 161/2022) of the National Health Surveillance Agency [29,30] for microbiological food standards for fresh and prepared vegetable products, with no specific standard for microgreens, it proves good hygiene conditions and correct handling of samples during harvest and storage, regardless of the packaging used. We also observed the absence of *E. coli* on all occasions.

Jablasone, Warriner and Griffiths [31] found *E. coli* O157:H7 in the internal tissues of watercress, lettuce, radish, and spinach seedlings but not in the mature plants' tissues. The pathogen preferentially colonized the epidermal root junctions since, during seed germination, the seed releases a mixture of carbohydrates and peptides that can attract neighboring bacteria in the rhizosphere.

Table 2. Total Enterobacteriaceae and *Escherichia coli* (log CFU/g) in arugula microgreens stored in different packages for 0 and 10 days.

Samples (S)	Time (T)		*p value*		
	0 days	10 days	S	T	S xT
Total Enterobacteriaceae					
Open	7.61 ± 0.60^{aA}	6.95 ± 0.44^{bA}			
Sealed	7.61 ± 0.60^{aA}	7.22 ± 0.54^{bA}	0.862	**0.039**	0.862
MAP	7.61 ± 0.60^{aA}	6.91 ± 0.38^{bA}			
Escherichia coli					
Open	Absent	Absent	-	-	-
Sealed	Absent	Absent	-	-	-

MAP	Absent	Absent	-	-	-

Open: open packaging, the plastic bag remains with the upper side open; Sealed: vacuum sealed packaging; MAP: modified atmosphere packaging. Different lowercase superscript in the same line indicates statistically significant difference for the time in the same package type by Tukey test ($p<0.05$). Different uppercase superscript in the same column indicates statistically significant difference for the package in the same time by Tukey test ($p<0.05$).

Chandra et al. [32] suggested that bacterial populations can easily grow on microgreens' delicate and immature tissue structure and may be stimulated by sugars and other organic molecules derived from the endosperm breakdown during germination. Post-harvest, respiration becomes the primary physiological process of the plant, which uses its own previously accumulated metabolic reserves. However, depending on the intensity of biochemical reactions, tissues can reach senescence faster, becoming more susceptible to moisture loss and the development of microorganisms [20].

Table 3 shows the microbial population of arugula microgreens after 0, 5 and 10 days of storage. The total aerobic mesophilic bacteria during the initial phase of storage was 7.3 ± 0.22 log CFU per g, with a statistical difference for subsequent storage days. On day 10 of storage, we can observe a statistically significant difference in relation to the packaging used. This fact can be explained by fermentative activity, which is a characteristic of biological oxidations in an oxygen-free environment, such as vacuum-sealed packaging. In this environment, pyruvic acid is converted into carbon dioxide and acetaldehyde [20].

Similar values of total aerobic mesophilic bacteria were found in the work of Paradiso et al. [5] with chicory microgreens. During the initial storage phase, the number was 6.69 ± 0.07 log CFU per g and 8.19 ± 0.07 log CFU/g in 10 days of storage. At their work, psychrotrophic microorganism counts were very similar to those of mesophilic microorganisms, which matches this study in 5 and 10 storage days.

Authors such as Chandra et al. [32] and Xiao et al. [33] identified the presence of mesophilic aerobic bacteria, molds and yeasts in these vegetables, with up to 10^7 CFU/g^{-1} and 10^5 CFU/g^{-1}, respectively. These levels can be considered potentially dangerous, both for the food's safety and its sensory quality and preservation capacity. It is worth mentioning that the International Commission on Microbiological Specifications for Foods (ICMSF) [31] states that foods with aerobic microorganism counts above 10^6 CFU/g typically show noticeable signs of spoilage, such as off-smells, off-tastes, and changes in appearance.

Table 3. Total count (log CFU/g) of mesophilic, psychrotrophilic, and molds and yeasts in arugula microgreens stored in different packages for 0, 5 and 10 days.

Samples (S)	Time (T)			*p value*		
	0 days	5 days	10 days	S	T	S x T
			Mesophilics			
Open	7.30 ± 0.22^{aA}	7.40 ± 0.40^{aA}	7.90 ± 0.26^{aB}			
Sealed	7.30 ± 0.22^{bA}	7.90 ± 0.23^{bA}	8.80 ± 0.42^{aA}	**0.008**	**<0.000**	**0.013**
MAP	7.30 ± 0.22^{aA}	7.90 ± 0.37^{aA}	7.70 ± 0.15^{aB}			
			Psychrotrophs			
Open	5.70 ± 0.10^{bA}	7.30 ± 0.16^{aB}	7.90 ± 0.58^{aA}			
Sealed	5.70 ± 0.10^{cA}	7.60 ± 0.05^{bAB}	8.60 ± 0.48^{aA}	**0.087**	**<0.000**	**0.003**
MAP	5.70 ± 0.10^{bA}	8.30 ± 0.50^{aA}	7.70 ± 0.15^{aA}			
			Yeasts and Molds			
Open	5.70 ± 0.06^{aA}	6.50 ± 0.28^{aB}	7.00 ± 1.09^{aB}			
Sealed	5.70 ± 0.06^{cA}	7.60 ± 0.09^{bAB}	8.90 ± 0.53^{aA}	**0.002**	**<0.000**	**0.001**
MAP	5.70 ± 0.06^{cA}	8.30 ± 0.83^{aA}	7.00 ± 0.01^{bB}			

Open: open packaging, the plastic bag remains with the upper side open; Sealed: vacuum sealed packaging; MAP: modified atmosphere packaging. Different lowercase superscript in the same line indicates statistically significant difference for the time in the same package type by Tukey test ($p<0.05$). Different uppercase superscript in the same column indicates statistically significant difference for the package in the same time by Tukey test ($p<0.05$).

The growth of yeasts and molds on buckwheat microgreens was relatively slow during the initial 8 days of storage (about 5.5 log CFU/g) and increased obviously from 8 to 12 days (up to 8.2 log CFU/g) storage in the study of Yan et al. [25]. Similar behavior was observed in the microgreens in this study, except the open packaging, which did not differ statistically.

FDA [34] requires that all foods in the "Time and Temperature Control for Safety" be maintained at a temperature not exceeding 5°C. Fresh-cut leafy green vegetables all belong to this category. Data in the study of Huang, Luo and Nou [35] showed that the proliferation of *Salmonella* and *L*. monocytogenes was more significantly impacted by long-term suboptimal refrigeration or frequent temperature fluctuation than short-term terminal exposure to higher temperatures in fresh-cut cantaloupe.

The environmental conditions that influence the development of plants in a hydroponic system are light, temperature, and humidity [36]. However, controlling climatic factors seemed challenging for microgreens growers, who occasionally treated seeds with hydrogen peroxide before planting to mitigate the potential proliferation of mold and pathogens [37]. But as reported by these authors, there is little evidence regarding the effectiveness of H_2O_2 for controlling microorganisms including pathogens on nonfood-contact and food-contact surfaces.

Despite their numerous benefits, the market availability of microgreens is limited, primarily due to their rapid senescence and highly perishable nature [19]. In table 4 we show the physical and chemical evaluation of microgreens up to the tenth day of storage.

According Kyriacou et al. [38], the optimal conditions for microgreen growth are a pH between 6.56 and 7.54. Unfortunately, it is the same range for the development of neutrophil bacteria. In this work, we could observe a drop in pH within seven days of storage, with a statistical difference between open and modified atmosphere packaging. This can be explained by the degradation of nitrogenous compounds present in the leaves of microgreens, releasing ammonia. Ammonia, when combined with water, resulting from cellular respiration, forms ammonium hydroxide, a weak base. However, the production of acids during cellular respiration often exceeds the production of bases, resulting in tissue acidification [20].

The increasing acidity at 5 days, which happened in this study, may be attributed to the biochemical conversion of fatty acids to acids over time [39]. After that, it is expected to decrease over time due to the plant's physiological processes.

In this study, the effect of time on the increase in soluble solids (SS) occurred on days 5 and 7, whereas the interaction with the MAP did not differ significantly in 10 days of storage. This can be attributed to differential utilization of metabolites in by respiration getting influenced by the permeabilities of the packaging material to gasses in this package [42].

Table 4. Physicochemical analysis of arugula microgreens stored in different packages for 0, 3, 5, and 10 days

Samples (S)	Time (T)					*p value*		
	0 days	3 days	5 days	7 days	10 days	S	T	S x T
pH								
Open	5.34±0.10^{bA}	6.39±0.04^{aA}	6.05±0.53^{aA}	4.54±0.16^{cA}	5.93±0.10^{aA}			
Sealed	5.34±0.10^{bA}	6.35±0.11^{aA}	6.24±0.82^{aA}	4.85±0.11^{cA}	6.29±0.06^{aA}	0.432	**<0.000**	0.908
MAP	5.34±0.10^{bA}	6.20±0.07^{aA}	6.12±0.87^{aA}	4.95±0.12^{cA}	6.26±0.23^{aA}			
Soluble solids								
Open	9.43±0.05^{bA}	9.26±0.05^{cB}	9.70±0.00^{aA}	9.66±0.05^{aA}	9.50±0.00^{bA}			
Sealed	9.43±0.05^{bA}	9.43±0.05^{bA}	9.70±0.00^{aA}	9.66±0.05^{aA}	9.46±0.05^{bA}	0.114	**<0.000**	**0.017**
MAP	9.43±0.05^{bA}	9.43±0.05^{bA}	9.66±0.05^{aA}	9.66±0.05^{aA}	9.56±0.05^{abA}			
Titratable acidity								
Open	2.46±0.92 ^{aA}	0.90±0.17^{bcA}	1.43±0.66^{bA}	0.63±0.15^{cA}	0.53±0.32^{cA}			
Sealed	2.46±0.92 ^{aA}	0.86±0.30^{bcA}	1.46±0.89^{bA}	0.66±0.05^{cA}	0.40±0.10^{cA}	0.988	**<0.000**	0.999
MAP	2.46±0.92 ^{aA}	1.03±0.25^{bcA}	1.50±0.70^{bA}	0.76±0.15^{cA}	0.23±0.05^{cA}			
Weight loss								
Open	-	8.93±2.02^{bA}	9.05±2.09^{bC}	15.03±1.36^{aB}	16.85±3.63^{aB}			
Sealed	-	7.15±2.43^{cA}	13.10±1.00^{bB}	15.02±2.38^{abB}	17.79±1.35^{aB}	**<0.000**	**<0.000**	**<0.000**
MAP	-	9.86±0.34^{dA}	16.44±1.76^{cA}	20.32±1.90^{bA}	24.14±1.27^{aA}			

Open: open packaging, the plastic bag remains with the upper side open; Sealed: vacuum sealed packaging; MAP: modified atmosphere packaging. Different lowercase superscript in the same line indicates statistically significant difference for the time in the same package type by Tukey test ($p<0.05$). Different uppercase superscript in the same column indicates statistically significant difference for the package in the same time by Tukey test ($p<0.05$).

Leafy vegetables are highly susceptible to water loss after harvest and the respiration and other senescence-related metabolic processes are the primary cause of postharvest loss [43]. In this work, the weight loss increased with increasing storage time, with no statistical difference ($p < 0.05$) for 7 and 10 days, but with a difference for MAP. Also, it was observed that, although the weight loss was greater in the MAP, the microgreens presented a better visual quality compared to those in the open packaging, since contact with the environment caused dehydration in the microgreens closest to the opening.

In the work of Patil et al. [21], the post-harvest treatment of ascorbic acid + citric acid along with MAP of broccoli microgreens package significantly ($p < 0.05$) suppressed the weight loss that helped to retain better firmness. According to Khan and Mittal [44], the efficiency of MAP depends upon multiple factors like appropriate gas composition, oxygen transmission rate (OTR), freshness, degree of processing of the product, product surface area, metabolism, respiration rate, microbial quality of produce, storage temperature, and relative humidity.

As Kou et al. [19] and Xiao et al. [36] explained, a favorable O_2/CO_2 balance and absence of anaerobic conditions that cause physiological damage to leaf tissue is required. When controlling these factors, the effect of storage temperature on the shelf life of microgreens appears to be more critical than the gas permeability of the packaging. At 5 °C of storage, the microgreens remained good for consumption up to ten days of analysis.

The chlorophyll content is important for the health benefits it offers and reflects on the appearance of the microgreens. Various shades of greenness in microgreens add to their aesthetic appeal [45]. In this work, the microgreens' total chlorophyll content (Table 5) ranged from 31.03±1.38 mg/100 g to 15.04±2.43 mg/100 g at 10 days in MAP. In general, the decline in chlorophylls can be observed from the third day onwards, with a statistical difference ($p < 0.05$). Chandra et al. [35] observed that polypropylene films, due to the more significant accumulation of CO_2, caused faster and more irreversible damage to the membrane compared to polyethylene, generating unpleasant odors. Furthermore, electrolyte leakage may contribute to faster yellowing, tissue senescence, and chlorophyll degradation of vegetables [36].

Similar values were found for total chlorophyll in the work of Kowitcharoen et al. [46], who analyzed some Brassicaceae microgreens such as, Rat-tailed radish and red cabbage (36.61 and 39.79 mg/100 g, respectively). In the study of Ghoora, Hanldipur & Srividya [47], the total chlorophyll of radish microgreens was 50.9 mg/100 g, but with a similar proportion of chlorophyll a and b (chlorophyll a approximately 2x higher) in relation to this work.

Color parameters are presented in Table 5. The L coordinate indicates lightness, which decreased significantly over the storage period, after 3 days. The a* coordinate, denoting greenness, become less negative signifying a decrease in ing samples. The b* value, representing yellowing, also showed a regressive incline over greenness over the storage period. However, no significant difference ($p < 0.05$) was observed in open-package same storage period for a* coordinate.

Table 5. Chlorophyll and color analysis of arugula microgreens stored in different packages for 0, 3, 5, 7, and 10 days

Samples	Time (T)					*p value*		
(S)	0 days	3 days	5 days	7 days	10 days	S	T	S x T
Chlorophyll a								
Open	19.67±0.64^{aA}	18.69±0.84^{aA}	17.47±1.22^{aA}	14.80±1.26^{bA}	12.38±0.73^{bA}			
Sealed	19.67±0.64^{aA}	13.96±0.30^{cB}	16.04±1.22^{bA}	14.08±0.03^{cA}	11.42±0.68^{dA}	**<0.000**	**<0.000**	**<0.000**
MAP	19.67±0.64^{aA}	17.62±0.39^{bA}	17.16±0.24^{bA}	10.88±0.22^{cB}	8.70±0.18^{dB}			
Chlorophyll b								
Open	11.35±2.02^{aA}	9.37±1.60^{bA}	8.59±1.32^{bA}	11.31±2.24^{bA}	10.00±0.83^{bA}			
Sealed	11.35±2.02^{aA}	6.77±1.07^{bA}	6.33±1.37^{bA}	5.91±0.07^{bA}	6.88±1.94^{bA}	**<0.000**	**<0.000**	0.720
MAP	11.35±2.02^{aA}	8.71±0.42^{bA}	6.17±1.51^{bA}	5.51±0.37^{bA}	6.34±2.32^{bA}			
Total chlorophyll								
Open	31.03±1.38^{aA}	28.07±0.93^{abA}	26.07±0.15^{bcA}	26.11±3.50^{bcA}	22.39±0.52^{cA}			
Sealed	31.03±1.38^{aA}	20.73±1.21^{bcB}	22.38±0.91^{bB}	19.99±0.10^{bcB}	18.31±1.26^{cB}	**<0.000**	**<0.000**	**<0.000**
MAP	31.03±1.38^{aA}	26.33±0.32^{bA}	25.79±1.72^{bA}	16.39±0.37^{cB}	15.04±2.43^{cB}			
Color L								
Open	53.41±6.56^{aA}	51.64±1.17^{bA}	44.30±1.96^{bcA}	44.62±0.03^{cA}	39.08±0.02^{cA}			
Sealed	53.41±6.56^{aA}	44.58±5.67^{bA}	41.50±1.14^{bcA}	41.90±0.03^{cA}	41.39±0.03^{cA}	0.222	**<0.000**	0.377
MAP	53.41±6.56^{aA}	45.35±3.17^{bA}	44.94±0.09^{bcA}	40.53±0.01^{cA}	39.83±0.29^{cA}			
Color a*								
Open	-14.82±1.50^{aA}	-11.23±3.82^{aA}	-12.39±0.01^{aA}	-11.12±0.03^{aB}	-9.93±0.02^{aC}	0.596	**<0.000**	**0.029**
Sealed	-14.82±1.50^{bA}	-11.82±2.99^{bA}	-13.86±1.69^{bA}	-11.08±0.01^{bB}	-6.43±0.01^{aA}			
MAP	-14.82±1.50^{cA}	-13.01±2.10^{cA}	-11.79±0.05^{bcA}	-7.75±0.01^{aA}	-9.15±0.03^{abB}			
Color b*								
Open	25.15±1.20^{aA}	22.35±3.84^{abA}	26.81±0.45^{aA}	18.66±0.25^{bcA}	15.52±0.02^{cA}	**0.002**	**<0.000**	**<0.000**
Sealed	25.15±1.20^{aA}	20.60±2.84^{abA}	22.49±3.25^{abA}	17.88±0.03^{bcB}	14.57±0.02^{cB}			
MAP	25.15±1.20^{aA}	24.18±1.54^{aA}	17.19±0.05^{bB}	15.56±0.02^{bcC}	14.71±0.21^{cB}			

Open: open packaging, the plastic bag remains with the upper side open; Sealed: vacuum sealed packaging; MAP: modified atmosphere packaging. Different lowercase superscript in the same line indicates statistically significant difference for the time in the same package type by Tukey test ($p<0.05$). Different uppercase superscript in the same column indicates statistically significant difference for the package in the same time by Tukey test ($p<0.05$).

Katsenios et al. [45] reported that a bright green color corresponds to the high-quality index for microgreens of green vegetable species, and yellowing suggests the product's quality deterioration. The green basil microgreens had similar behavior in the work of Ciriello et al. [46]: luminosity (L; 48.06), greenness (a*; −17.30), and yellowness (b*; 28.92). El-Nakhel et al. [47] and Petropoulos et al. [48] reported that nutrient availability may affect the color of microgreens' leaves and, thus, improve the visual quality of the final product.

According to sensory analysis results (Table 6), the sealed package showed a significant difference in 5 and 7 days in all attributes when compared to the third day. This result can be proven by the visual quality of the microgreens in this packaging, as shown in Supplementary Figure 1 (FS1). In contrast, we can observe a similarity between the open and MAP treatment (Supplementary Figure 2 (FS2)). In the study of Dhaka et al. [49] mustard microgreen was the first to undergo deterioration, and its visual quality showed some changes after four days.

Table 6. Sensory evaluation of arugula microgreens stored in different packages for 3, 5, and 7 days

Samples (S)	Time (T)			*p value*		
	3 days	5 days	7 days	S	T	S x T
Appearance						
Fresh	7.55±1.63^{abA}	7.43±1.50^{bA}	8.20±0.96^{aA}	**<0.00**	**<0.00**	**<0.00**
Open	7.66±1.54^{aA}	7.46±1.37^{aA}	7.37±1.39^{aA}			
Sealed	7.63±1.57^{aA}	3.07±1.72^{bB}	3.27±1.89^{bB}			
MAP	7.60±1.28^{aA}	7.35±1.42^{aA}	7.42±1.52^{aA}			
Odor						
Fresh	6.63±1.63^{aAB}	5.89±1.55^{aA}	6.57±1.66^{aA}	**<0.000**	**<0.000**	**0.009**
Open	6.50±1.90^{aAB}	5.84±1.49^{aA}	6.00±1.63^{aA}			
Sealed	6.33±1.86^{aB}	4.33±1.82^{bB}	4.57±2.12^{bB}			
MAP	7.25±1.31^{aA}	5.74±1.75^{bA}	6.40±1.56^{bA}			
Color						
Fresh	7.80±1.41^{abA}	7.38±1.42^{bA}	8.22±0.97^{aA}			
Open	7.88±1.48^{aA}	7.43±1.41^{aA}	7.62±1.35^{aA}	**<0.000**	**<0.000**	**<0.000**
Sealed	7.80±1.45^{aA}	4.07±1.97^{bB}	4.50±2.18^{bB}			
MAP	7.62±1.06^{aA}	7.41±1.39^{aA}	7.32±1.54^{aA}			
Texture						
Fresh	7.50±1.39^{aA}	7.74±1.31^{aA}	7.82±1.15^{aA}			
Open	7.45±1.56^{aA}	7.61±1.33^{aA}	7.62±1.35^{aA}	**<0.000**	**<0.000**	**<0.000**
Sealed	7.26±1.84^{aA}	4.33±2.46^{bB}	3.80±2.15^{bB}			
MAP	7.58±1.27^{aA}	7.41±1.44^{aA}	7.60±1.48^{aA}			
Taste						
Fresh	6.32±2.35^{aB}	6.64±2.06^{aA}	7.07±1.99^{aA}			
Open	6.03±2.29^{aB}	6.64±1.85^{aA}	6.82±2.06^{aA}	**<0.000**	0.156	**<0.000**
Sealed	6.25±2.48^{aB}	4.74±2.33^{bB}	3.77±2.42^{bB}			
MAP	7.37±1.46^{aA}	6.66±1.72^{aA}	6.80±1.84^{aA}			
Global acceptance						

Fresh	6.76±1.88^{bAB}	6.79±1.55^{bA}	7.67±1.26^{aA}			
Open	6.55±2.00^{aB}	6.84±1.64^{aA}	7.20±1.34^{aA}	**<0.000**	**0.002**	**<0.000**
Sealed	6.56±1.98^{aB}	4.53±2.24^{bB}	3.80±2.17^{bB}			
MAP	7.60±1.21^{aA}	6.82±1.47^{bA}	7.25±1.37^{abA}			

Open: open packaging, the plastic bag remains with the upper side open; Sealed: vacuum sealed packaging; MAP: modified atmosphere packaging. Different lowercase superscript in the same line indicates statistically significant difference for the time in the same package type by Tukey test ($p<0.05$). Different uppercase superscript in the same column indicates statistically significant difference for the package in the same time by Tukey test ($p<0.05$).

In the descriptive analysis evaluated in the work of Bafumo et al. [50], the evaluators identified rocket and watercress microgreens as the most astringent, standing out for pronounced bitterness and sourness, distinguishing them from the rest. Assessed microgreens evaluated by Caracciolo et al. [51] were consistently greater for appearance scores than those concerning texture and flavor. Their statistical analysis indicated that the observed differences in microgreens acceptability depended on two main sensory dimensions experienced through the consumer test: astringency/sourness and bitterness.

In relation to the acceptability index (Figure 1), as expected, the fresh sample obtained the best result (91.38%) for color, followed by appearance (91.11%) and texture (86.94%). It is noteworthy that, regardless of the sealed packaging in 5 and 7 days, all acceptability indexes of all treatments reached values above 60%, which is considered satisfactory.

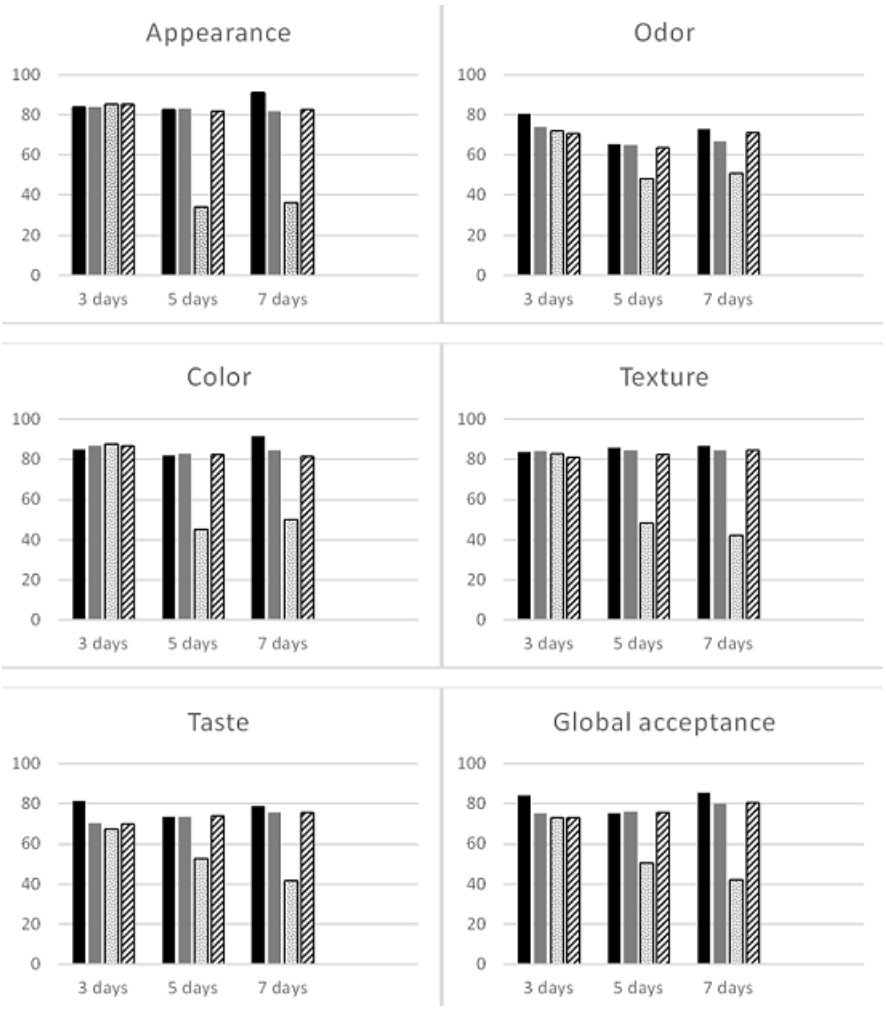


Figure 1. Acceptability index of sensory attributes of fresh (in black), open (in gray), sealed (with dots) and MAP (with stripes) arugula microgreens.

4. Conclusions

Microgreens stored in all packaging were safe for consumption within ten days. Regarding physico-chemical parameters, open packaging proved to be promising, with less weight loss and slower chlorophyll degradation, with maintenance of the greenness. The sensory analysis demonstrated that the microgreens stored in the vacuum-sealed packaging showed a worsening in quality from the fifth day onwards, for all attributes. However, the MAP presented good scores, with a better visual quality, similar to the fresh microgreens.

Further analysis can be done to enhance microgreens shelf life. Also, we hope that the findings of the study will contribute to this field and inspire investigation into effective methods for food packaging and preservation.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, **Figure S1**: Visual quality of fresh (without packaging), open (open packaging, the plastic bag remains with the upper side open), sealed (vacuum sealed packaging) and MAP (modified atmosphere packaging) arugula microgreens in seven days of storage; **Figure S2**: Sensory analysis radar chart of fresh (without packaging), open (open packaging, the plastic bag remains with the upper side open), sealed (vacuum sealed packaging) and MAP (modified atmosphere packaging) arugula microgreens.

Author Contributions: M. R. Komeroski and T. Beninca are PhD in Food Science and Technology and were research fellow at Capes. K. A. Portal is nutrition student at UFRGS and was scientific initiation scholarship holder. T. V. Klug is professor of Postgraduate Program in Science and Food Technology at Farroupilha Federal Institute. P. Malheiros, S. H. Flores, and A. O. Rios are full professor of Postgraduate Program in Food Science and Technology at the Federal University of Rio Grande do Sul. All of the authors were involved with the whole aspects of the paper: conception of the idea, framework writing, interpretation, and/or execution.

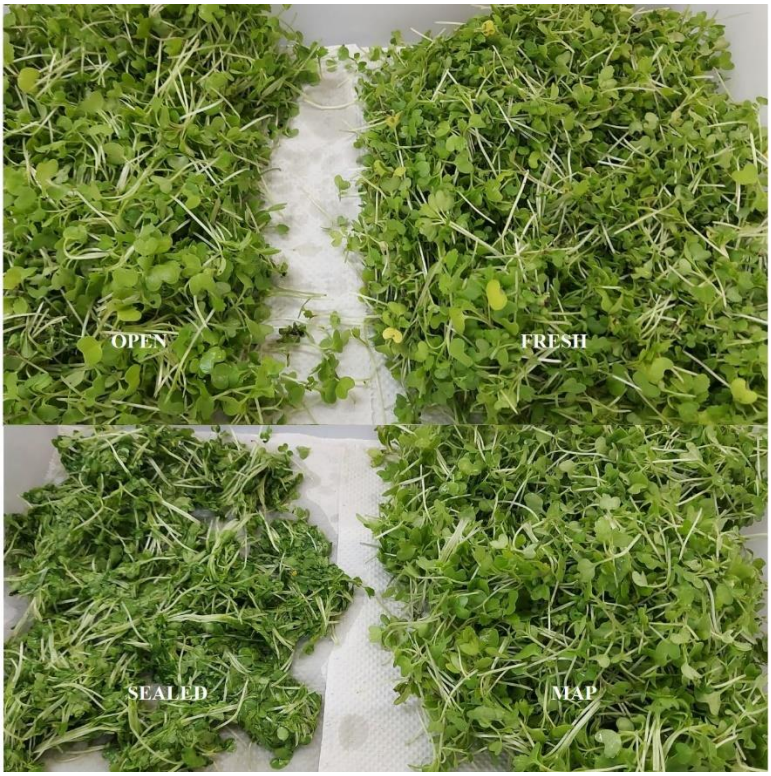
Funding: This research received no external funding.

Data Availability Statement: Data sharing is not applicable to this article.

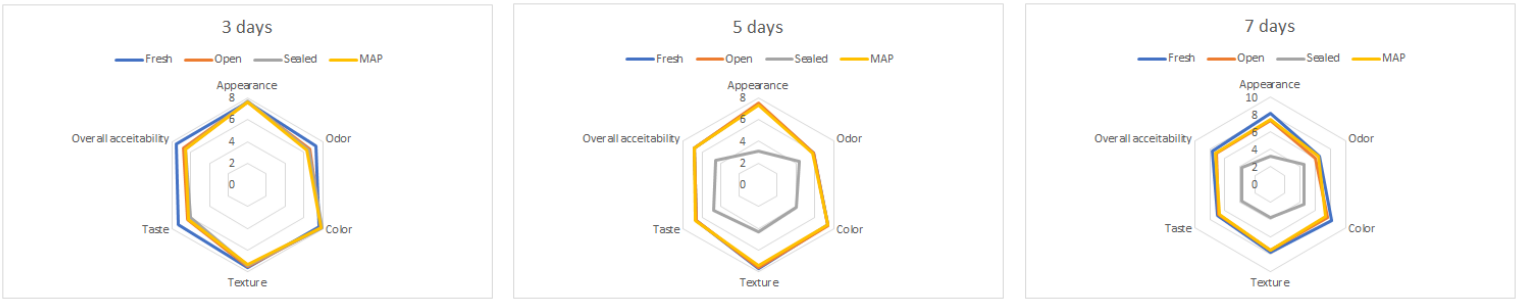
Acknowledgments: "Capes" for the study fellowship.

Conflicts of Interest: The authors declare no conflict of interest.

Supplementary materials



FS 1. Visual quality of fresh (without packaging), open (open packaging, the plastic bag remains with the upper side open), sealed (vacuum sealed packaging) and MAP (modified atmosphere packaging) arugula microgreens in seven days of storage.



FS 2. Sensory analysis radar chart of fresh (without packaging), open (open packaging, the plastic bag remains with the upper side open), sealed (vacuum sealed packaging) and MAP (modified atmosphere packaging) arugula microgreens.

References

1. Alruwaih, N. A., & Yaylayan, V. A. (2017). Comparative evaluation of bioactive compounds in lyophilized and tray-dried rocket (*Eruca sativa*). *Journal of Food Processing and Preservation*, 41(5), e13205.
2. Xiao, Z., Rausch, S., Luo, Y., Sun, J., Yu, L., Wang, Q., Chein, P., Stommel, J. (2019). Microgreens of Brassicaceae: Genetic diversity of phytochemical concentrations and antioxidant capacity. *LWT*, 101: 731-737.
3. Zhang, Y., Xiao, Z., Ager, E., Kong, L., Tan, L. (2021). Nutritional quality and health benefits of microgreens, a crop of modern agriculture. *Journal of Future Foods*, 1(1): 58-66.
4. Turner, E. R., Luo, Y., & Buchanan, R. L. (2020). Microgreen nutrition, food safety, and shelf life: A review. *Journal of food science*, *85*(4), 870-882.
5. Paradiso, V. M., Castellino, M., Renna, M., Gattullo, C. E., Calasso, M., Terzano, R., Santamaria, P. (2018). Nutritional characterization and shelf-life of packaged microgreens. *Food & Function*, 9, 5629-5641.
6. Butkutė, B., Taujenis, L., & Norkevičienė, E. (2018). Small-seeded legumes as a novel food source. Variation of nutritional, mineral and phytochemical profiles in the chain: Raw seeds-sprouted seeds-microgreens. *Molecules*, *24*(1), 133.
7. Di Gioia, F., Renna, M., Santamaria, P. Sprouts, microgreens and "Baby Leaf" vegetables. In *Minimally Processed Refrigerated Fruits and Vegetables*; Yildiz, F., Wiley, R.C., Eds.; Springer: Boston, MA, USA, 2017; pp. 403–432.
8. Johnson, S., Prenni, J., Heuberger, A., Isweiri, H., Chaparro, J., Newman, S., Uchanski, M., Omerigic, H., Michell, K., Bunning, M., Foster, M., Thompson, H., & Weir, T. (2021). Comprehensive evaluation of metabolites and minerals in 6 microgreen species and the influence of maturity. *Current developments in nutrition, 5(2),* nzaa180.
9. Kopsell, D., Pantanizopoulos, N., Sams, C., & Kopsell, D. (2012). Shoot tissue pigment levels increase in "Florida Broadleaf" mustard (*Brassica juncea* L.) microgreens following high light treatment. *Scientia Horticulturae, 140,* 96–99.
10. Di Gioia, F., Mininni, C., & Santamaria, P. (2015). Come coltivare i micro-ortaggi—How to grow microgreens—Cómo cultivar micro-hortalizas. In F. Di Gioia & P. Santamaria (Ed.), *Microgreens* (pp. 51–79). Bari, Italy: Eco-logica editore.
11. Kyriacou, M., Rouphael, Y. Di Gioia, F., Kyratzis, A., Serio, F., Renna, M., & de Pascale, S. (2016). Micro-scale vegetable production and the rise of microgreens. *Trends in Food Science and Technology, 57,* 103–115.
12. Renna, M., Castellino, M., Leoni, B., Paradiso, V. M., & Santamaria, P. (2018). Microgreens production with low potassium content for patients with impaired kidney function. *Nutrients*, 10(6), 675.
13. Ebert, A., Wu, T., & Yang, R. Y. (2014). Amaranth sprouts and microgreens—A homestead vegetable production option to enhance food and nutrition security in the rural–urban continuum. In: *Proceedings of the regional symposium on sustaining small-scale vegetable production and marketing systems for food and nutrition security.* (pp. 233–244). Taiwan: AVRDC Publication.
14. Komeroski, M. R., Portal, K. A., Comiotto, J., Klug, T. V., Flores, S. H., & Rios, A. D. O. (2023). Nutritional quality and bioactive compounds of arugula (*Eruca sativa* L.) sprouts and microgreens. *International Journal of Food Science & Technology*, 58(10), 5089-5096.

15. Riggio, G., Wang, Q., Kniel, K., & Gibson, K. (2019). Microgreens—A review of food safety considerations along the farm to fork continuum. *International Journal of Food Microbiology*, 290, 76-85.
16. Wright, K. M. and Holden, N. J. (2018). Quantification and colonisation dynamics of *Escherichia coli* O157: H7 inoculation of microgreens species and plant growth substrates. *International Journal of Food Microbiology*, 273, 1-10.
17. Turner, E. R., Luo, Y., & Buchanan, R. L. (2020). Microgreen nutrition, food safety, and shelf life: A review. *Journal of food science*, *85*(4), 870-882. Di Gioia, F., De Bellis, P., Mininni, C., Santamaria, P., & Serio, F. (2016). Physicochemical, agronomical and microbiological evaluation of alternative growing media for the production of rapini (*Brassica rapa* L.) microgreens. *Journal of the Science of Food and Agriculture, 97(4)*, 1212–1219.
18. Mir SA, Shah MA, Mir MM. (2016). Microgreens: Production, shelf life, and bioactive components. *Critical Reviews in Food Science and Nutrition*, 57: 2730–2736.
19. Sharma, A., Hazarika, M., Heisnam, P., Pandey, H., Devadas, V. S., Singh, D., ... & Kartha, B. D. (2023). Influence of storage conditions, packaging, post-harvest technology, nanotechnology and molecular approaches on shelf life of microgreens. *Journal of Agriculture and Food Research*, 14, 100835.
20. Kou, L., Luo, Y., Yang, T., Xiao, Z., Turner, E. R., Lester, G. E. & Camp, M. J. (2013). Postharvest biology, quality and shelf life of buckwheat microgreens. *LWT-Food Science and Technology*, 51(1), 73-78.
21. Patil, M., Sharma, S., Sridhar, K., Anurag, R. K., Grover, K., Dharni, K., ... & Sharma, M. (2024). Effect of postharvest treatments and storage temperature on the physiological, nutritional, and shelf-life of broccoli (*Brassica oleracea*) microgreens. *Scientia Horticulturae*, 327, 112805.
22. Chitarra, M. I. F. and Chitararra, A. B. Pós-colheita de frutas e hortaliças: fisiologia e manuseio. 2. ed. rev. amp. Lavras: Universidade Federal de Lavras, 2005. 783 p.
23. Silva, N.; Junqueira, V., Silveira, N., Taniwaki, M., Gomes, R., Okazaki, M., Iamanaka, B. *Manual de Métodos de Análise Microbiológica de Alimentos e Água*. 6 ed. São Paulo: Blucher, 2021.
24. Barbosa, A.D., Alexandre, B., Tondo, E.C., Malheiros, P. S. (2022). Microbial survival in gourmet hamburger thermally processed by different degrees of doneness. *International Journal of Gastronomy and Food Science*, 28: 100501.
25. AOAC. *Official Methods of Analysis of AOAC International*, 16th ed.; AOAC International: Rockville, MD, USA, 1995; Volume 1.
26. Xiao, Z., Lester, G. E., Park, E., Saftner, R. A., Luo, Y., & Wang, Q. (2015). Evaluation and correlation of sensory attributes and chemical compositions of emerging fresh produce: Microgreens. *Postharvest Biology and Technology*, 110, 140-148.
27. Yan, H., Li, W., Chen, H., Liao, Q., Xia, M., Wu, D. & Zhao, J. (2022). Effects of Storage Temperature, Packaging Material and Wash Treatment on Quality and Shelf Life of Tartary Buckwheat Microgreens. *Foods*, 11(22), 3630.
28. Wellburn, A. R., & Lichtenthaler, H. (1984). Formulae and program to determine total carotenoids and chlorophylls a and b of leaf extracts in different solvents. In *Advances in Photosynthesis Research: Proceedings of the VIth International Congress on Photosynthesis, Brussels, Belgium, August 1–6, 1983 Volume 2* (pp. 9-12). Springer Netherlands.

29. Viana, L. T. (2009). Sensory analysis in the food industry. Revista Instituto Laticínios Cândido Tostes; 64(366), 12–21.
30. Priti, Sangwan S, Kukreja B, Mishra GP, Dikshit HK, Singh A, et al. (2022) Yield optimization, microbial load analysis, and sensory evaluation of mungbean (*Vigna radiata* L.), lentil (*Lens culinaris* subsp. culinaris), and Indian mustard (*Brassica juncea* L.) microgreens grown under greenhouse conditions. *PLoS ONE*, 17(5), e0268085.
31. International Commission on Microbiological Specifications for Foods – ICMSF. (2011). *Microorganisms in Foods 8: Microbiological testing in food safety management*. New York: Kluwer Academic.
32. BRASIL. 2022. RESOLUÇÃO - RDC Nº 724, DE 01 DE JULHO DE 2022. Available at: https://antigo.anvisa.gov.br/documents/10181/2718376/RDC_724_2022_.pdf/33c61081-4f32-43c2-9105-c318fa6069ce accessed in July 2024.
33. BRASIL. 2022. INSTRUÇÃO NORMATIVA N° 161, DE 01 DE JULHO DE 2022. Available at: https://antigo.anvisa.gov.br/documents/10181/2718376/IN_161_2022_.pdf/b08d70cb-add6-47e3-a5d3-fa317c2d54b2 accessed in July 2024.
34. Jablasone, J., Warriner, K., & Griffiths, M. (2005). Interactions of *Escherichia coli* O157: H7, Salmonella typhimurium and Listeria monocytogenes plants cultivated in a gnotobiotic system. *International journal of food microbiology*, 99(1), 7-18.
35. Chandra, D., Kim, J. G. and Kim, Y. P. (2012). Changes in microbial population and quality of microgreens treated with different sanitizers and packaging films. *Horticulture, Environment and Biotechnology*, 53, 32–40.
36. Xiao, Z., Luo, Y., Lester, G. E., Kou, L., Yang, T., & Wang, Q. (2014). Postharvest quality and shelf life of radish microgreens as impacted by storage temperature, packaging film, and chlorine wash treatment. *LWT - Food Science and Technology*, 55(2), 551–558.
37. Kyriacou, M. C., El-Nakhel, C., Pannico, A., Graziani, G., Soteriou, G. A., Giordano, M. & Rouphael, Y. (2020). Phenolic constitution, phytochemical and macronutrient content in three species of microgreens as modulated by natural fiber and synthetic substrates. *Antioxidants*, 9(3), 252.
38. Huang, J., Luo, Y., & Nou, X. (2015). Growth of Salmonella enterica and *Listeria monocytogenes* on fresh-cut cantaloupe under different temperature abuse scenarios. *Journal of Food Protection*, 78(6), 1125-1131.
39. Rusu, T., Moraru, P. I., & Mintas, O. S. (2021). Influence of environmental and nutritional factors on the development of lettuce (*Lactuca sativa* L.) microgreens grown in a hydroponic system: A review. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 49(3), 12427.
40. FDA. 2009. Recommendations for the Temperature Control of Cut Leafy Greens during Storage and Display in Retail Food Establishments. Available at: https://www.fda.gov/media/78982/download accessed in August 2024.
41. Hamilton, A. N., Fraser, A. M., & Gibson, K. E. (2023). Barriers to implementing risk management practices in microgreens growing operations in the United States: Thematic analysis of interviews and survey data. *Food Control*, 152, 109836.
42. Dalal, N., & Siddiqui, S. (2019). Effect of chemical treatment, storage and packaging on physico-chemical properties of sunflower microgreens. *International Journal of Chemical Studies*, 7, 1046-1050.
43. Gómez PA, Egea-Gilabert C, Giménez A, Benaissa RR, Amoruso F, Signore A, Gallegos-Cedillo VM, Ochoa J, Fernández JA. (2023). Biodegradable Food Packaging of Wild

Rocket (*Diplotaxis tenuifolia* L. [DC.]) and Sea Fennel (*Crithmum maritimum* L.) Grown in a Cascade Cropping System for Short Food Supply Chain. *Horticulturae*, 9(6):621.
44. Khan, M. A. M., & Mittal, A. (2020). Modified atmosphere packaging technique: An overview. In *International Conference on* 507 *Recent Advances in Engineering and Science*.
45. De La Fuente, B., López-García, G., Máñez, V., Alegría, A., Barberá, R., & Cilla, A. (2019). Evaluation of the bioaccessibility of antioxidant bioactive compounds and minerals of four genotypes of Brassicaceae microgreens. *Foods*, 8(7), 250.
46. Kowitcharoen, L., Phornvillay, S., Lekkham, P., Pongprasert, N., & Srilaong, V. (2021). Bioactive composition and nutritional profile of microgreens cultivated in Thailand. *Applied Sciences*, 11(17), 7981.
47. Ghoora, M. D., Haldipur, A. C., & Srividya, N. (2020). Comparative evaluation of phytochemical content, antioxidant capacities and overall antioxidant potential of select culinary microgreens. *Journal of Agriculture and Food Research*, *2*, 100046.
48. Katsenios, N., Christopoulos, M. V., Kakabouki, I., Vlachakis, D., Kavvadias, V., & Efthimiadou, A. (2021). Effect of pulsed electromagnetic field on growth, physiology and postharvest quality of kale (*Brassica oleracea*), wheat (*Triticum durum*) and spinach (*Spinacia oleracea*) microgreens. *Agronomy*, 11(7), 1364.
49. Ciriello, M., Formisano, L., El-Nakhel, C., Zarrelli, A., Giordano, M., De Pascale, S. & Rouphael, Y. (2023). Iodine biofortification of four microgreens species and its implications for mineral composition and potential contribution to the recommended dietary intake of iodine. *Scientia Horticulturae*, 320, 112229.
50. El-Nakhel, C., Pannico, A., Graziani, G., Kyriacou, M. C., Gaspari, A., Ritieni, A., ... & Rouphael, Y. (2021). Nutrient supplementation configures the bioactive profile and production characteristics of three Brassica L. microgreens species grown in peat-based media. *Agronomy*, 11(2), 346.
51. Petropoulos, S. A., El-Nakhel, C., Graziani, G., Kyriacou, M. C., & Rouphael, Y. (2021). The effects of nutrient solution feeding regime on yield, mineral profile, and phytochemical composition of spinach microgreens. *Horticulturae*, 7(7), 162.
52. Dhaka, A. S., Dikshit, H. K., Mishra, G. P., Tontang, M. T., Meena, N. L., Kumar, R. R. & Praveen, S. (2023). Evaluation of growth conditions, antioxidant potential, and sensory attributes of six diverse microgreens species. *Agriculture*, 13(3), 676.
53. Bafumo, R. F., Alloggia, F. P., Ramirez, D. A., Maza, M. A., Fontana, A., Moreno, D. A., & Camargo, A. B. (2024). Optimal brassicacea family microgreens from a phytochemical and sensory perspective. *Food Research International*, 193, 114812.
54. Caracciolo, F., El-Nakhel, C., Raimondo, M., Kyriacou, M. C., Cembalo, L., De Pascale, S., & Rouphael, Y. (2020). Sensory attributes and consumer acceptability of 12 microgreens species. *Agronomy*, 10(7), 1043.

CAPÍTULO 5

6. DISCUSSÃO GERAL

A produção de microgreens em escala doméstica ou comercial demonstra ser uma nova tendência de mercado. É uma prática rápida, sustentável, econômica e segura que necessita ser mais explorada, principalmente no Brasil.

Em um primeiro momento foi realizado uma busca extensiva na literatura para caracterizar e apresentar um panorama geral do estudo dos microgreens pelo mundo. Verificou-se que a grande maioria dos artigos publicados estuda a família Brassicaceae, não só pelo seu ciclo anual, mas também por serem vegetais muito consumidos, com sabor característico. Em nosso artigo, categorizamos os microgreens dessa família de acordo com a apresentação do vegetal estudado: florais e folhosos. Demonstrou-se que brotos e microgreens podem ter níveis mais altos de compostos bioativos em relação aos mesmos vegetais maduros, principalmente pela etapa da germinação, com as mudanças fisiológicas e bioquímicas que ocorrem, e por não passarem por tratamentos agressivos na etapa de pós-colheita.

Apesar de não se conseguir determinar a influência exata dos sistemas de cultivo no conteúdo bioativo desses vegetais, o uso de luz, parece ser uma estratégia positiva a ser adotada a fim de estimular a produção de compostos bioativos, variando intensidade e fotoperíodo de acordo com a planta cultivada.

Pela primeira vez, pelo menos até o presente momento, foi realizada uma comparação entre brotos e microgreens de um mesmo vegetal, seguindo condições iguais de cultivo e submentendo ambos as mesmas análises, a fim de elucidar diversas questões em relação à composição centesimal e conteúdo de compostos bioativos, já que esses dois estágios de maturação vegetal são confundidos e/ou erroneamente utilizados como sinônimos.

Como ainda não há legislação específica para cultivo e armazenamento de microgreens este trabalho também analisou a vida útil desses vegetais em três diferentes embalagens. Embora as três formas de embalagem sejam seguras para consumo em até 10 dias de armazenamento - o que demostra que o sistema de cultivo escolhido

(hidropônico do tipo flutuante) atende os padrões de higiene pretendidos e pode ser reproduzido -, em relação à qualidade visual e, portanto, potencial comercial desses microgreens, nosso estudo demonstrou que a embalagem selada à vácuo não é uma escolha interessante. A concentração de umidade oriunda do processo de respiração dessas plantas ficou retida nesse tipo de embalagem, fazendo-os murchar quando comparadas com as outras embalagens.

7. CONCLUSÃO

Os microgreens de rúcula apresentam um alto valor nutricional, com teores significativos de proteínas e fibras, além de compostos bioativos que superam os níveis encontrados nos vegetais maduros. A comparação entre brotos e microgreens revelou que os brotos têm um teor superior de proteínas e fibras, enquanto os microgreens possuem compostos bioativos importantes, como os flavonoides. A análise de vida útil mostrou que os microgreens permanecem microbiologicamente seguros e nutricionalmente valiosos por até 10 dias quando armazenados em condições adequadas, sendo a embalagem de atmosfera modificada a mais indicada devido a melhor qualidade visual do produto.

APÊNDICE A - Folder a ser divulgado em mídias sociais e e-mails

PARTICIPE!

ANÁLISE SENSORIAL DE MICROGREENS DE RÚCULA

DATA: xx/xx/xxxx

HORA: xx:xx

LOCAL: Laboratório de Análise Sensorial - ICTA

Trabalho para obtenção do grau de Doutora
Aluna: Marina Rocha Komeroski
Orientador: Alessandro Rios de Oliveira

APÊNDICE B - Ficha para avaliação sensorial das amostras

Você está recebendo microgreens de rúcula orgânicos cultivados em hidroponia. Por favor, analise cada amostra separadamente e aplique notas na tabela abaixo para cada atributo avaliado de acordo com o critério abaixo:

(1) Desgostei muitíssimo
(2) Desgostei muito
(3) Desgostei moderadamente
(4) Desgostei ligeiramente
(5) Nem gostei, nem desgostei
(6) Gostei ligeiramente
(7) Gostei moderadamente
(8) Gostei muito
(9) Gostei muitíssimo

Atributos	**Amostras**			
	060	**330**	**530**	**700**
Aparência				
Odor				
Cor				
Textura				
Sabor				
Aceitação global				

APÊNDICE C - Termo de Consentimento Livre e Esclarecido

Projeto: **Avaliação da qualidade nutricional, bioativa e tecnológica de microgreens e brotos de rúcula**

Pesquisadora: Marina Komeroski
Participantes envolvidos: Alunos e servidores da UFRGS
Data: ___/___/___

I. Justificativa e Objetivos:

A busca por alternativas que contenham alta qualidade sensorial, mas também um bom perfil nutricional faz com que haja pesquisas de novos ingredientes para se investigar essas atuais exigências. Neste sentido, microgreens de rúcula estão sendo avaliados pois parecem fornecer maiores quantidades de compostos bioativos, além de serem fáceis de cultivar, produzidos de forma orgânica e de rápida colheita. Esse trabalho tem como objetivo avaliar esses vegetais submetidos à diferentes embalagens e em diferentes dias de armazenamento sob refrigeração, tanto para benefícios nutricionais, bem como benefícios funcionais, incluindo sabor e textura.

II. Os procedimentos a serem utilizados:

Esse consentimento está relacionado com a avaliação sensorial de microgreens de rúcula submetidos à diferentes embalagens em diferentes dias de armazenamento sob refrigeração. Os participantes serão convidados por folders virtuais em mídias sociais e e-mails para participar da avaliação sensorial no Laboratório de Análise Sensorial do ICTA/UFRGS. O deslocamento até a Faculdade é custeado pelos próprios participantes. Os participantes receberão as amostras simultaneamente, codificadas com 3 dígitos aleatórios, um copo de água para limpeza das papilas gustativas e uma ficha sensorial com uma escala hedônica de 9 pontos para se julgar os atributos: aparência, cor, sabor, textura e aceitação global. Todos os procedimentos duram, aproximadamente, 10 minutos.

III. Desconfortos e riscos:

Essa análise será realizada somente com a concordância e a disponibilidade do participante em contribuir com o estudo, caso contrário será prontamente respeitado. Acredita-se, assim, que esse estudo seja de risco reduzido, pois as amostras serão oferecidas dentro do prazo de validade, refrigeradas e microbiologicamente seguras. A pesquisadora fica responsável de prontamente encaminhar qualquer participante ao serviço de saúde se o mesmo apresentar qualquer problema relacionado a essa análise sensorial. Os participantes terão o direito de abandonar este estudo, caso se sintam prejudicados ou tenham se arrependido de participar, e em qualquer momento terão

liberdade de solicitar novas informações. Este trabalho terá total sigilo quanto aos resultados que venham a envolver o avaliador.

IV. Os benefícios que se pode obter:

Será avaliada a melhor forma de processamento em relação ao aspecto sensorial pelos avaliadores, associando embalagens seladas, abertas e/ou com atmosfera modificada mantidas sob refrigeração em diferentes dias de armazenamento. Dessa forma, pretende-se verificar qual a embalagem garante o melhor resultado, não apenas sensorialmente agradável, mas também nutricionalmente positivo, seguro e inócuo.

V. Garantia de privacidade:

Os seus dados de identificação serão mantidos em sigilo e as informações colhidas serão analisadas estatisticamente, serão guardadas por um período de cinco anos e podem ser publicadas posteriormente em alguma revista científica. Afirmo que a sua participação poderá ser suspensa a qualquer momento caso você deseje, sem prejuízo para a sua pessoa.

VI. Garantia de resposta a qualquer pergunta e liberdade de abandonar a pesquisa:

Eu, _____ fui informado dos objetivos do estudo realizado pela pesquisadora Marina Komeroski, portanto concordo em participar deste projeto. Sei que em qualquer momento poderei solicitar novas informações e modificar minha decisão se assim eu desejar. Caso tiver novas perguntas sobre este estudo, posso recorrer às pesquisadoras no telefone (51) 33089787.
Declaro que tenho conhecimento do presente Termo de Consentimento.

_____ _____

Assinatura do participante Assinatura do pesquisador

REFERÊNCIAS

Andrews, Z., Fateh, E., Shaver, E., Lee, F., Hernandez, A., Rivera, M., & Nolan, D. (2019). Evaluating the Yield of Helianthus annuus Microgreens When Treated with Sodium Hypochlorite and Other Factors. *Journal of Student-Scientists' Research*, 1.

Ahmed, J., Al-Salman, F., & Almusallam, A. S. (2013). Effect of blanching on thermal color degradation kinetics and rheological behavior of rocket (Eruca sativa) puree. *Journal of Food Engineering*, 119, 660–667.

Alloggia FP, Bafums RF, Ramirez DA, Maza MA, Camargo AB (2023) *Brassicaceae* microgreens: A novel and promissory source of sustainable bioactive compounds. Current Research in Food Science, 6, 100480.

Alruwaih, N. A., & Yaylayan, V. A. (2017). Comparative evaluation of bioactive compounds in lyophilized and tray-dried rocket (*Eruca sativa*). *Journal of Food Processing and Preservation*, 41(5), e13205.

AOAC. *Official Methods of Analysis of AOAC International*, 16th ed., AOAC International: Rockville, MD, USA, 1995; Volume 1.

AOAC: Association of Official Analytical Chemists. *Official methods of analysis of AOAC international*. 20th ed., AOAC International, 2006.

Barbosa, A.D., Alexandre, B., Tondo, E.C., Malheiros, P. S. (2022). Microbial survival in gourmet hamburger thermally processed by different degrees of doneness. *International Journal of Gastronomy and Food Science*, 28: 100501.

Bhaswant M, Shanmugam DK, Miyazawa T, Abe C, Miyazawa T (2023) Microgreens - A comprehensive review of bioactive molecules and health benefits. Molecules, 28, 867.

Bell, L., & Wagstaff, C. (2014). Glucosinolates, myrosinase hydrolysis products, and flavonols found in rocket (*Eruca sativa* and *Diplotaxis tenuifolia*). *Journal of Agricultural and Food Chemistry*, 62, 4481–4492.

Bennett, R. N., Rosa, E. A. S., Mellon, F. A., & Kroon, P. A. (2006). Ontogenic profiling of glucosinolates, flavonoids, and other secondary metabolites in *Eruca sativa* (salad rocket), *Diplotaxis erucoides* (wall rocket), *Diplotaxis tenuifolia* (wild rocket), and *Bunias orientalis* (turkish rocket). *Journal of Agricultural and Food Chemistry*, 54, 4005–4015.

Björkman, M., Klingen, I., Birch, A., Bones, A., Bruce, T., Johansen, T. J., Stewart, D. (2011). Phytochemicals of Brassicaceae in plant protection and human health – Influences of climate, environment and agronomic practice. *Phytochemistry*, 72(7), 538–556.

Bulgari, R., Baldi, A., Ferrante, A., & Lenzi, A. (2016). Yield and quality of basil, Swiss chard, and rocket microgreens grown in a hydroponic system. *New Zealand Journal of Crop and Horticultural Science*, 45(2), 119–129.

Butkutė, B., Taujenis, L., & Norkevičienė, E. (2018). Small-seeded legumes as a novel food source. Variation of nutritional, mineral and phytochemical profiles in the chain: Raw seeds-sprouted seeds-microgreens. *Molecules*, *24*(1), 133.

Castro-Ibáñez, I., Gil, M. and Allende, A. (2017). Ready-to-eat vegetables: Current problems and potential solutions to reduce microbial risk in the production chain. *LWT-Food Science and Technology*, 85, 284-292.

Chandra, D., Kim, J. G. and Kim, Y. P. (2012). Changes in microbial population and quality of microgreens treated with different sanitizers and packaging films. *Horticulture, Environment and Biotechnology*, 53, 32–40.

Cheng, D. M., Pogrebnyak, N., Kuhn, P., Krueger, C. G., Johnson, W. D., Raskin, I. (2014). Development and phytochemical characterization of high polyphenol red lettuce with anti-diabetic properties. *PLOS ONE*, 9(3), e91571.

Chitarra, M. I. F. and Chitararra, A. B. *Pós-colheita de frutas e hortaliças: fisiologia e manuseio*. 2. ed. rev. amp. Lavras: Universidade Federal de Lavras, 2005. 783 p.

Choe, U., Yu, L. & Wang, T. (2018). The science behind microgreens as an exciting new food for the 21th century. *Journal of Agricultural and Food Chemist*ry, 66, 11519-11530.

Corleto, K. A., Singh, J., Jayaprakasha, G. K., & Patil, B. S. (2018). Storage Stability of Dietary Nitrate and Phenolic Compounds in Beetroot (*Beta vulgaris*) and Arugula (*Eruca sativa*) Juices. *Journal of Food Science*, 83(5), 1237–1248.

Dayarathna, N. N., Gama-Arachchige, N. S., Damunupola, J. W., Xiao, Z., Gamage, A., Merah, O., & Madhujith, T. (2023). Effect of storage temperature on storage life and sensory attributes of packaged mustard microgreens. *Life*, *13*(2), 393.

Di Gioia F, Mininni C and Santamaria P. (2015). How to grow microgreens, in *Microgreens*, ed. by Di Gioia F and Santamaria P. Eco-logica, Bari, pp. 51–79.

Di Gioia, F., Mininni, C., & Santamaria, P. (2015b). Come coltivare i micro-ortaggi—How to grow microgreens—Cómo cultivar micro-hortalizas. In F. Di Gioia & P. Santamaria (Ed.), *Microgreens* (pp. 51–79). Bari, Italy: Eco-logica editore.

Di Gioia, F., De Bellis, P., Mininni, C., Santamaria, P., & Serio, F. (2016). Physicochemical, agronomical and microbiological evaluation of alternative growing media for the production of rapini (Brassica rapaL.) microgreens. *Journal of the Science of Food and Agriculture*, 97(4), 1212–1219.

Di Gioia, F., Renna, M. and Santamaria, P. (2017). Sprouts, Microgreens and "Baby Leaf" Vegetables. In*:* Food Engineering Series, pp. 403–432.

Drygaś B, Piechowiak T, Balawejder M, Matłok N, Kreczko J, Puchalski C (2024) The eliciting effect of aqueous extracts from *Ascophyllum nodosum* algae on the cultivation of arugula (*Eruca sativa* Mill.) microgreens. Sustainability, 16, 7436.

D'Imperio M, Bonelli L, Mininni C, Renna M, Montesano FF, Parente A, Serio F (2024) Soilless cultivation systems to produce tailored microgreens for specific nutritional needs. Journal of the Science of Food and Agriculture, 104, 3371-3380.

Ebert AW (2022) Sprouts and microgreens - Novel food sources for healthy diets. Plants, 11, 571.

Fusari CM, Nazareno MA, Locatelli DA, Fontana A, Beretta V, Camargo AB (2020) Phytochemical profile and functionality of *Brassicaceae* species. Food Bioscience, 36, 100606.

Gui, M., He, H., Li, Y., Chen, X., Wang, H., Wang, T., & Li, J. (2018). Effect of UV-B treatment during the growth process on the postharvest quality of mung bean sprouts (*Vigna radiata*). *International Journal of Food Science & Technology*, *53*(9), 2166-2172.

Jablasone, J., Warriner, K., & Griffiths, M. (2005). Interactions of *Escherichia coli* O157:H7, Salmonella Typhimurium and Listeria monocytogenes plants cultivated in a gnotobiotic system. *International Journal of Food Microbiology*, 99, 7–18.

Kathi, S., Laza, H., Singh, S., Thompson, L., Li, W., & Simpson, C. (2022). Increasing vitamin C through agronomic biofortification of arugula microgreens. *Scientific Reports*, *12*(1), 13093.

Kyriacou, M.C., Rouphael, Y., Di Gioia, F., Kyratzis, A., Serio, F., Renna, M., De Pascale, S., Santamaria, P. (2016). Micro-scale vegetable production and the rise of microgreens. *Trends in Food Science and Technology*, 57, 103–115.

Kyriacou, M. C., El-Nakhel, C., Graziani, G., Pannico, A., Soteriou, G. A., Giordano, M., Rouphael, Y. (2018). Functional quality in novel food sources: genotypic variation in the

nutritive and phytochemical composition of thirteen microgreens species. *Food Chemistry*.

Kou, L., Luo, Y., Yang, T., Xiao, Z., Turner, E. R., Lester, G. E. & Camp, M. J. (2013). Postharvest biology, quality and shelf life of buckwheat microgreens. *LWT-Food Science and Technology*, 51(1), 73-78.

Koukounaras, A., Siomos, A., & Sfakiotakis, E. (2010). Effects of 6-ba treatments on yellowing and quality of stored rocket (*Eruca sativa* Mill.) Leaves. Journal of Food Quality, 33(6), 768–779.

Liu, R. H. (2013). Dietary Bioactive Compounds and Their Health Implications. *Journal of Food Science*, 78(s1), A18–A25.

Lerner B L, Strassburger AS, Schäfer G (2024). Cultivation of arugula microgreens: seed densities and electrical conductivity of nutrient solution in two growing seasons. Bragantia, 83, e20230183.

Mercadante, A. Z., Britton, G., Rodriguez-Amaya, D. B. (1998). Carotenoids from yellow passion fruit (*Passiflora edulis*). *Journal of Agricultural and Food Chemistr*y, 46, 4102-4106.

Mir, S. A., Shah, M. A., & Mir, M. M. (2016). Microgreens: Production, shelf life, and bioactive components. *Critical Reviews in Food Science and Nutrition*, 57(12), 2730–2736.

NAS-IOM. (2001). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenun, Nickel, Silicon, Vanadium, and Zinc. National A ed. Washington, DC: [s.n.].

Ntsoane, M. L., Manhivi, V. E., Shoko, T., Seke, F., Sultanbawa, Y., & Sivakumar, D. (2024). Brassica microgreens cabbage (*Brassica oleracea*), radish (*Raphanus sativus*) and rocket (*Eruca vesicaria* L.) Cav: application of red-light emitting diodes lighting

during postharvest storage and in vitro digestion on bioactive compounds and antioxidant activity. *International Journal of Food Science & Technology*, *59*(3), 1432-1442.

Paradiso, V. M., Castellino, M., Renna, M., Gattullo, C. E., Calasso, M., Terzano, R., Santamaria, P. (2018). Nutritional characterization and shelf-life of packaged microgreens. *Food & Function*, 9, 5629-5641.

Pasini, F., Verardo, V., Caboni, M. F., & D'Antuono, L. F. (2012). Determination of glucosinolates and phenolic compounds in rocket salad by HPLC-DAD–MS: Evaluation of *Eruca sativa* Mill. and *Diplotaxis tenuifolia* L. genetic resources. *Food Chemistry*, 133(3), 1025–1033.

Pasini, F., Verardo, V., Cerretani, L., Caboni, M. F., & D'Antuono, L. F. (2011). Rocket salad (*Diplotaxis* and *Eruca* spp.) sensory analysis and relation with glucosinolate and phenolic content. Journal of the Science of Food and Agriculture, 91(15), 2858–2864.

Patil, M., Sharma, S., Sridhar, K., Anurag, R. K., Grover, K., Dharni, K., ... & Sharma, M. (2024). Effect of postharvest treatments and storage temperature on the physiological, nutritional, and shelf-life of broccoli (*Brassica oleracea*) microgreens. *Scientia Horticulturae*, 327, 112805.

Pinto, E., Almeida, A. A., Aguiar, A. A., & Ferreira, I. M. (2015). Comparison between the mineral profile and nitrate content of microgreens and mature lettuces. *Journal of Food Composition and Analysis*, *37*, 38-43.

Renna, M., Di Gioia, F., Leoni, B., Mininni, C., & Santamaria, P. (2016). Culinary Assessment of Self-Produced Microgreens as Basic Ingredients in Sweet and Savory Dishes. *Journal of Culinary Science & Technology*, 15(2), 126–142.

Riggio, G., Wang, Q., Kniel, K., & Gibson, K. (2019). Microgreens—A review of food safety considerations along the farm to fork continuum. *International Journal of Food Microbiology*, 290, 76-85.

Rodrigues, E., Mariutti, L. R. B., Mercadante, A. Z. Carotenoids and Phenolic Compounds from Solanum sessilif lorum, an Unexploited Amazonian Fruit, and Their Scavenging Capacities against Reactive Oxygen and Nitrogen Species. *Journal of Agricultural and Food Chemistry*, 61(12), 3022–3029.

Rufino, M. (2007). Metodologia Científica: Determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH. *Ministério da Agricultura, Pecuária e Abastecimento*, 23(2), 1–4.

Samuolienė, G., Viršilė, A., Brazaitytė, A., Jankauskienė, J., Sakalauskienė, S., Vaštakaitė, V. & Duchovskis, P. (2017). Blue light dosage affects carotenoids and tocopherols in microgreens. *Food Chemistry, 228*, 50-56.

Silva, N.; Junqueira, V., Silveira, N., Taniwaki, M., Gomes, R., Okazaki, M., Iamanaka, B. *Manual de Métodos de Análise Microbiológica de Alimentos e Água*. 6 ed. São Paulo: Blucher, 2021.

Silveira, A. C., Baeza, A., & Escalona, V. H. (2015). Effect of Ultraviolet-C Radiation Combined with Unconventional Atmosphere Packaging on the Quality of Fresh-Cut Arugula (*Eruca sativa* Mill.). *Journal of Food Safety*, 35(4), 523–532.

Singh, M., Nara, U., Rani, N., Pathak, D., Kaur, K., & Sangha, M. K. (2023). Comparison of mineral composition in microgreens and mature leaves of celery (*Apium graveolens* L.). *Biological Trace Element Research, 201*(8), 4156-4166.

Tassi, É. M. M., Duarte, R. M. T., & Amaya-Farfan, J. (2018). Partial nutrient characterization of arugula (rocket - Eruca sativa L.) and the effect of heat treatment on its lipoxidase activity. *Brazilian Journal of Food Technology*, 21(0).

Teng Z, Luo Y, Pearlstein DJ, Wheeler RM, Johnson CM, Wang Q, Fonseca JM (2023) Microgreens for home, commercial, and space farming: A comprehensive update of the

most recent developments. Annual Review of Food Science and Technology, 14, 539-562.

Tomás-Callejas, A. & Artés, F. (2010). Low UV-C illumination for keeping overall quality of fresh-cut watermelon. *Postharvest Biology and Technology*, 55, 114–120.

Turner, E. R., Luo, Y., & Buchanan, R. L. (2020). Microgreen nutrition, food safety, and shelf life: A review. *Journal of food science*, *85*(4), 870-882.

Treadwell, D. D., Hochmuth, R., Landrum, L., Laughlin, W. (2010). Microgreens: A New 451 Specialty Crop. University of Florida IFAS Extension, HS1164.

USDA – United States Department of Agriculture (2014) Specialty greens pack a nutricional punch. AgResearch Magazine. Available at http://agresearchmag.ars.usda.gov/2014/jan/greens accessed in June 2023.

Viana, L. T. (2009). Sensory analysis in the food industry. Revista Instituto Laticínios Cândido Tostes; 64(366), 12–21.

Wellburn, A. R., & Lichtenthaler, H. (1984). Formulae and program to determine total carotenoids and chlorophylls a and b of leaf extracts in different solvents. In *Advances in Photosynthesis Research: Proceedings of the VIth International Congress on Photosynthesis, Brussels, Belgium, August 1–6, 1983 Volume 2* (pp. 9-12). Springer Netherlands.

Xiao, Z., Lester, G., Luo, Y. (2012). Assessment of vitamin and carotenoid concentrations of emerging food products: edible microgreens. *Journal of Agricultural and Food Chemistry*, 60, 7644-7651.

Xiao, Z., Lester, G. E., Park, E., Saftner, R. A., Luo, Y., & Wang, Q. (2015). Evaluation and correlation of sensory attributes and chemical compositions of emerging fresh produce: Microgreens. *Postharvest Biology and Technology*, 110, 140-148.

Xiao, Z., Codling, E. E., Luo, Y., Nou, X., Lester, G. E., & Wang, Q. (2016). Microgreens of Brassicaceae: Mineral composition and content of 30 varieties. *Journal of Food Composition and Analysis*, 49, 87–93.

Xiao, Z., Rausch, S., Luo, Y., Sun, J., Yu, L., Wang, Q., Stommel, J. R. (2019). Microgreens of Brassicaceae: Genetic diversity of phytochemical concentrations and antioxidant capacity. *LWT*, 101, 731-737.

Yan, H., Li, W., Chen, H., Liao, Q., Xia, M., Wu, D. & Zhao, J. (2022). Effects of Storage Temperature, Packaging Material and Wash Treatment on Quality and Shelf Life of Tartary Buckwheat Microgreens. *Foods*, 11(22), 3630.

Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. (2000). Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *New England Journal of Medicine*, 342, 154–60.

Zhang, G., Xu, Z., Gao, Y., Huang, X., Zou, Y., Yang, T. (2015). Effects of Germination on the Nutritional Properties, Phenolic Profiles, and Antioxidant Activities of Buckwheat. *Journal of Food Science*, 80, H1111–H1119.

Zhang, X., Bian, Z., Yuan, X., Chen, X., & Lu, C. (2020). A review on the effects of light-emitting diode (LED) light on the nutrients of sprouts and microgreens. *Trends in Food Science & Technology*, 99, 203-216.