Universidade do Estado do Rio de Janeiro
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Estudo sobre germinação de sementes de chia (Salvia hispânica L.): qualidade microbiológica, perfil lipídico, compostos fenólicos e atividade antioxidante

Rio de Janeiro
2023

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Dissertação apresentada, como requisito parcial para obtenção do título de Mestre, ao Programa de Pós-Graduação em Alimentação, Nutrição e Saúde, da Universidade do Estado do Rio de Janeiro.

Orientadora: Prof.ª Dra.Roberta Fontanive Miyahira

Coorientadora: Profª. Dra Lilia Zago

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RESUMO

SALGADO, Vanessa dos Santos Chiappetta Nogueira. *Estudo sobre germinação de sementes de chia (Salvia hispanica L.)*: qualidade microbiológica, perfil lipídico, compostos fenólicos e atividade antioxidante. 2023. 105 f. Dissertação (Mestrado em Alimentação, Nutrição e Saúde) – Instituto de Nutrição, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2023.

A chia ganhou popularidade devido às suas propriedades nutricionais e funcionais. A germinação das sementes de chia tem sido estudada como uma forma de melhorar ainda mais essas propriedades. No entanto, o consumo de sementes germinadas cruas tem sido associado a surtos de doenças transmitidas por alimentos. Portanto, além de avaliar a qualidade nutricional das sementes germinadas, este estudo também analisará a qualidade microbiológica, visando identificar possíveis patógenos que possam estar presentes. As análises da composição centesimal foram realizadas segundo os métodos oficiais. O perfil de ácidos graxos foi determinado por cromatografia gasosa acoplada a espectrômetro de massa. Para determinação do teor de fenólicos totais foi utilizado o método Folin-Ciocalteau. A identificação dos compostos fenólicos foi feita por HPLC. Foi determinada a capacidade antioxidante usando os métodos FRAP, ABTS e o DPPH. As análises microbiológicas foram realizadas para coliformes totais, E. coli, Aeróbios mesófilos e Bacillus cereus, Salmonella spp. e Listeria monocytogenes. Os resultados foram avaliados estatisticamente por análise de variância (ANOVA), as médias comparadas pelo teste Tukey ($p \leq 0,05$). Durante a germinação, os brotos de chia apresentaram um aumento significativo na concentração de minerais e proteínas, enquanto houve uma redução no teor total de lipídios, sem alterar a relação ômega-6:ômega-3 presentes. Observou-se uma diminuição significativa no conteúdo fenólico total dos brotos de chia à medida que o tempo de germinação aumentou, porém houve um aumento significativo na quantidade de ácido rosmarínico. Além disso, os brotos de chia demonstraram um aumento significativo no potencial antioxidante quando comparados às sementes de chia cruas. Não foram detectadas cepas de Salmonella spp., E. coli e Listeria monocytogenes nas amostras analisadas. No entanto, foi observada nos brotos uma alta carga microbiana de coliformes totais e aeróbios mesófilos mesmo após os processos de sanitização com hipoclorito de sódio. Bacillus cereus foi encontrado em amostras de brotos de chia. Em conclusão, os resultados deste estudo evidenciaram que a germinação das sementes de chia é um processo de curta duração, acessível economicamente e de fácil execução, que promove melhorias significativas na composição nutricional das sementes. A carga microbiana permaneceu elevada mesmo após o processo de sanitização. É fundamental que órgãos competentes desenvolvam e ampliem a divulgação de diretrizes de boas práticas de produção para o cultivo seguro de brotos em ambiente doméstico com o intuito de incentivar sua produção de maneira segura do ponto de vista microbiológico.

Palavras-chave: Chia. Broto. Composição nutricional. Sanitização de alimentos. Alimento seguro.

ABSTRACT

SALGADO, Vanessa dos Santos Chiappetta Nogueira. *Study on germination of chia (Salvia hispanica L.) seeds*: microbiological quality, lipid profile, phenolic compounds and antioxidant activity. 2023. 105 f. Dissertação (Mestrado em Alimentação, Nutrição e Saúde) – Instituto de Nutrição, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2023.

Chia has gained popularity due to its nutritional and functional properties. The germination of chia seeds has been studied as a way to further enhance these properties. However, the consumption of raw sprouted seeds has been associated with outbreaks of foodborne illnesses. Therefore, in addition to evaluating the nutritional quality of germinated seeds, this study will also analyze their microbiological quality to identify possible pathogens that may be present. The proximate composition analyses were performed according to official methods. The fatty acid profile was determined by gas chromatography coupled with mass spectrometry. The total phenolic content was determined using the Folin-Ciocalteau method. The identification of phenolic compounds was done by HPLC. The antioxidant capacity was determined using the FRAP, ABTS, and DPPH methods. Microbiological analyses were performed for total coliforms, E. coli, mesophilic aerobes, Bacillus cereus, Salmonella spp., and Listeria monocytogenes. The results will be statistically evaluated by analysis of variance (ANOVA), and means were compared using the Tukey test ($p \leq 0.05$). During germination, chia sprouts showed a significant increase in mineral and protein concentration, while the total lipid content decreased without altering the omega-6:omega-3 ratio present. There was a significant decrease in the total phenolic content of chia sprouts as germination time increased, but there was a significant increase in the amount of rosmarinic acid. Additionally, chia sprouts exhibited a significant increase in antioxidant potential compared to raw chia seeds. No strains of Salmonella spp., E. coli, or Listeria monocytogenes in the analyzed samples were detected. However, there was a high microbial load of total coliforms and mesophilic aerobes in the sprouts even after the sanitization processes with sodium hypochlorite. Bacillus cereus was found in samples of chia sprouts. In conclusion, the results of this study demonstrate that the germination of chia seeds is a short-duration, economically accessible, and easily executable process that significantly improves the nutritional composition of the seeds. The microbial load remained high even after the sanitization process. It is crucial for competent authorities to develop and expand the dissemination of guidelines for safe sprout production in domestic settings in order to encourage its production in a microbiologically safe way.

Keywords: Chia. Sprout. Nutritional composition. Food sanitation. Food safety.

RESUMEN

SALGADO, Vanessa dos Santos Chiappetta Nogueira. *Estudio sobre la germinación de semillas de chía (Salvia hispanica L.)*: calidad microbiológica, perfil lipídico, compuestos fenólicos y actividad antioxidante. 2023. 105 f. Dissertação (Mestrado em Alimentação, Nutrição e Saúde) – Instituto de Nutrição, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2023.

La chía ha ganado popularidad debido a sus propiedades nutricionales y funcionales. La germinación de las semillas de chía se ha estudiado como una forma de mejorar aún más estas propiedades. Sin embargo, el consumo de semillas germinadas crudas se ha asociado con brotes de enfermedades transmitidas por alimentos. Por lo tanto, además de evaluar la calidad nutricional de las semillas germinadas, este estudio también analizará la calidad microbiológica con el objetivo de identificar posibles patógenos presentes. Los análisis de composición centesimal se realizaron según métodos oficiales. El perfil de ácidos grasos se determinó mediante cromatografía de gases acoplada a espectrometría de masas. El contenido fenólico total se determinó utilizando el método Folin-Ciocalteau. La identificación de compuestos fenólicos se realizó mediante HPLC. La capacidad antioxidante se determinó utilizando los métodos FRAP, ABTS y DPPH. Se realizaron análisis microbiológicos para coliformes totales, E. coli, aerobios mesófilos, Bacillus cereus, Salmonella spp. y Listeria monocytogenes. Los resultados se evaluaron estadísticamente mediante análisis de la varianza (ANOVA) y las medias se compararán mediante la prueba de Tukey ($p \leq 0,05$). Durante la germinación, los brotes de chía mostraron un aumento significativo en la concentración de minerales y proteínas, mientras que hubo una reducción en el contenido total de lípidos Sin alterar la relación omega-6:omega-3 restantes. Se observó una disminución significativa en el contenido fenólico total de los brotes de chía a medida que aumentaba el tiempo de germinación, pero hubo un aumento significativo en la cantidad de ácido rosmarínico. Además, los brotes de chía demostraron un aumento significativo en el potencial antioxidante en comparación con las semillas de chía crudas. No se detectaron cepas de Salmonella spp., E. coli y Listeria monocytogenes en las muestras analizadas. Sin embargo, se observó una alta carga microbiana de coliformes totales y aerobios mesófilos en los brotes. Se encontró Bacillus cereus en muestras de brotes de chía incluso después de los procesos de higienización con hipoclorito sódico. En conclusión, los resultados de este estudio muestran que la germinación de las semillas de chía es un proceso de corta duración, económicamente accesible y fácil de realizar, que promueve mejoras significativas en la composición nutricional de las semillas. La carga microbiana permaneció alta incluso después del proceso de saneamiento. Es fundamental que las autoridades competentes desarrollen y amplíen la difusión de pautas de buenas prácticas de producción para el cultivo seguro de brotes en entornos domésticos con el fin de fomentar su producción de forma microbiológicamente segura.

Palabras clave: Chia. Germinado. Composición nutricional. Saneamiento de alimentos. Alimento seguro.

PRINCIPAIS ACHADOS

Esses achados fornecem uma nova perspectiva sobre a utilização dos brotos de chia como uma opção de um alimento com elevado valor nutricional. Por meio da germinação, é possível potencializar os benefícios das sementes, como, o aumento de minerais, proteínas, ácido rosmarínico, capacidade antioxidantes, bem como a manutenção da qualidade lipídica, uma vez que não houve alteração na relação ômega-6:ômega-3 em relação a semente de chia crua. No entanto, é importante ressaltar a importância de garantir a segurança microbiológica durante o processo. A implementação de diretrizes de boas práticas de produção e descontaminação eficaz é essencial para minimizar os riscos de crescimento microbiano. Com esse conhecimento, espera-se que órgãos competentes possam desenvolver e divulgar orientações específicas para o cultivo seguro de brotos em ambiente doméstico. Assim, os consumidores poderão se beneficiar com a produção e consumo dos brotos de chia de forma confiável e segura.

LISTA DE ABREVIATURAS E SIGLAS

ABTS	ácido 2,2'-azinobis-3-etilbenzotiazolina-6-sulfônico
AOAC	Association of Official Analytical Chemists
BLEB	Base de Caldo de Enriquecimento Listeria Tamponado
DPPH 2,2	difenil-1-picrilhidrazil
E. coli	Escherichia Coli
FDA	Food and Drug Association
FRAP	Ferric Reducing Antioxidant Power
HE	Ágar Entérico de Hectoen
HPLC	Cromatografia Líquida de Alta Eficiência
IA	Índice de Aterogenicidade
IFSH	Institute for Food Safety and Health
IOM	Instituto de Medicina
IT	Índice de Trombogenicidade
LIA	Ágar de Ferro e Lisina
MNP	Número mais provável
MUFA	Ácidos graxos monoinsaturados
ORAC	Capacidade de Absorção de Radicais de Oxigênio
OXA	Ágar de Oxford
PCA	Agar Padrão de Contagem
PUFA	Ácidos graxos poli-insaturados
SC	Caldo Selenito Cistina
SS	Semente sanitizada
SSA	Sprout Safety Alliance
SSP	Brotos sanitizado
TSI	Ágar Tríplice Açúcar Ferro
TT	Caldo Tetrationato
UFA	Ácidos graxos insaturados
USP	Brotos não sanitizado
USS	Semente não sanitizada
XLD	Ágar Xilose Lisina

LISTA DE ILUSTRAÇÕES

SUMÁRIO

1 **INTRODUÇÃO**

Salvia hispanica L., também conhecida como chia, é uma planta herbácea, pertencente à família Lamiaceae, nativa das regiões montanhosas do oeste e centro do México e da Guatemala (Coelho et al. 2014).

A chia apresenta um elevado teor de proteínas, fibras, lipídeos, além de vitaminas e minerais, além de ser fonte de compostos bioativos, que possuem elevada atividade antioxidante, podendo então ser considerada um alimento funcional (Hrnčič et al., 2020). A chia apresenta benefícios à saúde e seu consumo tem sido associado à menor incidência de doenças crônicas não transmissíveis (Kaur & Bains, 2020; Kulczyński et al., 2019; Marcinek & Krejpcio, 2017).

Em busca de uma vida mais saudável, a população em geral está optando por alimentos *in natura* e uma alimentação mais baseada em plantas. Por isso, a demanda por alimentos saudáveis cultivados em sistemas naturais sem o uso de agrotóxicos vem aumentando (Benincasa et al., 2019; Lynch et al., 2018).

O cultivo de brotos comestíveis demanda pouco consumo de água, é um processo simples, de baixo custo e não requer solo, sendo possível sua realização em ambiente doméstico (Geng et al., 2022; Miyahira & Antunes, 2021; Warriner, 2014). Estudos têm demonstrado que a germinação de sementes de chia resulta em aumento do teor e qualidade proteica, de fibras totais, aumento do teor de compostos fenólicos totais e aumento da capacidade antioxidante (Abdel-Aty et al., 2021; Beltrán-Orozco et al., 2020; Gómez-Favela et al., 2017). Em relação ao perfil de ácidos graxos, Cabrera-Santos et al (2021) pesquisou as alterações que ocorrem durante a embebição de semente de chia e os resultados mostraram que as maiores concentrações de ácidos palmítico, esteárico, linoleico e α-linolênico foram observadas a 20°C.

Apesar da melhora dos valores nutricionais das sementes após a produção de brotos, pode ocorrer o crescimento de patógenos, uma vez que as condições em que ocorre a germinação são propícias para a proliferação de microrganismos que possam estar presentes nas sementes (Harvey et al., 2017). Outro fator contribuinte é que geralmente os brotos não sofrem qualquer tratamento de sanitização ou de redução de carga microbiana (Harvey et al., 2017). A contaminação de brotos por patógenos microbianos é uma grande preocupação, nas últimas décadas, em diversos países, e o consumo de sementes germinadas cruas é reconhecida

como uma importante causa de doenças transmitidas por alimentos (Ding et al., 2013; Harvey et al., 2017; Miyahira & Antunes, 2021; Yang et al., 2013).

Sendo assim, o objetivo principal deste trabalho consiste em avaliar a qualidade nutricional e microbiológica das sementes de chia durante o processo de germinação, por meio de análises e testes específicos, investigando as alterações ocorridas na composição nutricional da semente de chia com o processo de germinação, assim como identificar a presença de microrganismos patogênicos que possam afetar a segurança do consumo desse alimento.

Esta dissertação é composta por três capítulos, escritos sob a forma de artigos científicos. O primeiro capítulo intitulado: "**Chia (*Salvia hispanica* L.) Seed Germination: a Brief Review**". É uma revisão bibliográfica que apresenta uma revisão abrangente da literatura sobre as propriedades nutricionais da chia e a importância da germinação no aprimoramento dessas propriedades, além da observação dos riscos microbiológicos em seu consumo. Este artigo foi publicado em 2022 na revista Plant Foods for Human Nutrition (ISSN:1573-9104), volume 77, páginas 485–494 (DOI: 10.1007/s11130-022-01011-z).

O segundo capítulo é um artigo científico que será submetido ao periódico Plant Foods for Human Nutrition (ISSN: 1573-9104), intitulado "**Centesimal composition, fatty acid profile, phenolic compounds, and antioxidant activity of raw and germinated chia (*Salvia hispanica* L.) seeds**". Este artigo consiste em uma análise abrangente sobre o processo de germinação das sementes de chia em dois tempos distintos (3 e 6 dias), explorando suas características nutricionais e comparando os resultados com a semente antes da germinação.

O terceiro capítulo faz parte de uma pesquisa mais ampla, um projeto guarda-chuva, no qual o objetivo foi avaliar a qualidade microbiológica de brotos de chia, trigo e lentilha em ambiente doméstico e a eficácia do processo de sanitização de brotos e sementes com hipoclorito de sódio. O artigo elaborado foi composto por dados das análises microbiológicas dos brotos de chia e dos brotos de trigo e lentilha. Os dois últimos brotos (trigo e lentilha) foram analisados pelas alunas Flávia Oliveira Brito e Paula Stephany de Melo Rodrigues e os resultados fizeram parte do Trabalho de Conclusão de Curso (TCC) das alunas sob orientação da professora Dra Roberta Fontanive Miyahira e minha coorientação. O artigo intitulado "**Effect of sanitization with sodium hypochlorite on microbiological quality in home production of lentil, wheat, and chia sprouts**" será submetido ao periódico International Journal of Food Microbiology (ISSN: 1879-3460).

2 **REFERENCIAL TEÓRICO**

2.1 **Aspectos gerais sobre chia**

Salvia hispanica L., também conhecida como chia, é uma planta herbácea, pertencente à família Lamiaceae (Coelho et al. 2014). A planta da chia produz sementes pequenas de coloração brancas e escuras que amadurecem no outono (Capitani et al., 2012). Segundo Salgado-Cruz et al., (2013), observou-se que as sementes de chia possuem forma elíptica ou ovóide, com seu comprimento variando de 1,8 a 2,5 mm e sua largura de 1,5 a 2,0 mm. Nativa das montanhas do oeste e centro do México e da Guatemala, anteriormente era cultivada pelos maias e astecas e tinha uma grande importância pois era utilizada como alimento, tintas, artesanato e até mesmo como medicamento (Kaur & Bains, 2020; Coelho et al., 2014). Atualmente, o cultivo comercial de sementes de chia está concentrado principalmente em países como México, Bolívia, Equador, Guatemala, Colômbia, Peru, Argentina e Paraguai (Kulczyński et al., 2019).

Durante a hidratação da semente de chia ocorre o processo de quebra da cutícula devido à perda de elasticidade, e os componentes celulares são extravasados em forma de gel (mucilagem), que permanece firmemente ligado à semente e é composto principalmente de fibra solúvel (Capitani et al., 2012). Considerando a composição química e física das sementes de chia, por sua formação de gel, estas são capazes de atuar como espessantes, gelificantes e estabilizantes, além de melhorar o valor nutricional de algumas preparações (Salgado-Cruz et al., 2013). Além desses usos, a chia é comumente consumida como broto de chia em salada, em bebidas, cereais e molho de salada, ou ainda é consumida crua (Mohd Ali et al., 2012).

Em um estudo com mucilagem de chia, o autor Feizi et al., (2021) avaliou uma formulação de sorvete em que o gel extraído de semente de chia substituiu o estabilizante comercial, concluindo que o sorvete apresentou consistência desejável, textura suave e sensação cremosa na boca, sem sensação de cristais de gelo, demonstrando o potencial que a chia pode apresentar no desenvolvimento de produtos alimentícios, tais como em sorvetes, iogurtes, pães e bolos (Borneo et al., 2010; Feizi et al., 2021; Fernandes & Salas-Mellado, 2017; Ribes et al., 2021).

2.1.1 Composição nutricional

A chia é composta em média por um teor de lipídeos de 30–33%, carboidratos de 26–41%, fibras totais de 18– 30%, proteínas de 15–25%, vitaminas, minerais, além de ser fonte de compostos bioativos, que possuem uma elevada atividade antioxidante, podendo então ser considerada como um alimento funcional (Hrnčič et al., 2020). Esses valores podem ser alterados segundo o local de cultivo, condições climáticas, época de ano, entre outros fatores (Hrnčič et al., 2020).

Comparado a outros grãos, a semente de chia se destaca pelo seu teor de proteínas, uma vez que contém cerca de 19,6% e nove aminoácidos essenciais, uma relevante composição de aminoácidos essenciais e não essenciais (Pal & Raj, 2020; Ullah et al., 2016). Um estudo de Segura-Campos (2019), que teve por objetivo isolar e caracterizar as proteínas de sementes de chia, encontrou 42,94% de glutelinas, 20,81% de albuminas, 17,3% de globulinas e 5,81% de prolaminas, apesar do teor de proteínas ser suscetível de acordo com a variedade, método de extração e outras variáveis. Em outro estudo de (Valdivia-López & Tecante (2015), constataram que a semente de chia continha grandes quantidades de ácido glutâmico, arginina e ácido aspártico.

As sementes de chia possuem um teor lipídico de 25 a 35%, são ricas em ácidos graxos poliinsaturados, principalmente ácido graxo linolênico que correspondem a 60% do teor total (Coelho et al., 2014). O conteúdo de ácido graxo linoleico é em média de 18 a 20% e em menores proporções estão os de ácidos graxos saturados como ácido palmítico e esteárico.

Entretanto, esses teores dependem do estágio de desenvolvimento da planta, pois o conteúdo de ácido graxo linolênico pode diminuir do estágio inicial ao estágio maduro em até 23% (Imran et al., 2016; Kulczyński et al., 2019; Mohd Ali et al., 2012; Coelho et al., 2014). Além disso, a semente de chia possui ótima razão entre ácidos ômega-6 e ômega-3, de aproximadamente 0,3:0,35 (Kulczyński et al., 2019). Esse dado sobre a chia é interessante, pois os ácidos graxos ômega-3 são importantes para auxiliar no sistema imunológico, diabetes, câncer, possuem também propriedades cardioprotetoras e hepatoprotetoras (Lara et al., 2021).

As fibras presentes na chia podem desempenhar um papel importante na prevenção de hipercolesterolemia, diabetes, câncer colorretal e obesidade (Lara et al., 2021). Além do mais, são grandes aliadas na regulação do trânsito intestinal, pois colaboram para o aumento do bolo fecal, auxiliando assim na função intestinal regular. As fibras possuem também efeito de saciedade, redução do índice glicêmico, entre outros (Capitani et al., 2012; Lara et al., 2021;

Marcinek & Krejpcio, 2017.). As sementes de chia contêm aproximadamente de 30-34 g de fibra alimentar em 100g, das quais a fibra insolúvel corresponde aproximadamente a 85-93%, enquanto a quantidade de fibra solúvel é aproximadamente de 7-15% (Kulczyński et al., 2019). A chia possui um destaque pelo seu teor de fibras em comparação à quinoa, linhaça e amaranto (Pal & Raj, 2020).

Em um estudo, comparando sementes de chia da região de Jalisco e Sinaloa, encontrou-se a média do teor de fibras solúveis e insolúveis de 6,5 e 33,85 g/100 g respectivamente (Reyes-Caudillo et al., 2008). O principal componente de fibras insolúveis encontrado na chia foi a lignina, em seguida foi encontrado também a presença de celulose e hemicelulose (Reyes-Caudillo et al., 2008). Enquanto que as fibras solúveis representam cerca de 6% das sementes de chia, composto principalmente por açúcares neutros, o que indica a presença de diversos carboidratos que formam a estrutura da mucilagem (Reyes-Caudillo et al., 2008).

Os antioxidantes são moléculas que impedem o processo de oxidação dos radicais livres que são prejudiciais à saúde. A semente de chia possui diferentes compostos com ação antioxidantes como tocoferóis, esteróis, ácido protocatecuico, ácido gálico, ácido p-cumárico, ácido cafeico, epicatequina, quercetina e kaempferol (Pal & Raj, 2020). O consumo de alimentos ricos em compostos bioativos está associado a um risco menor de doenças crônicas não transmissíveis (Oliveira-Alves et al., 2017). Martínez-Cruz & Paredes-López (2014), ao analisar o teor de compostos fenólicos totais e atividade antioxidante na semente de chia, relataram que os compostos fenólicos totais foram 1,8 vezes maiores do que encontrado por outros autores. Nesse mesmo estudo foram identificados os seguintes compostos fenólicos na semente de chia: ácido rosmarínico, ácido protocatecuico e ácido cafeico. A atividade antioxidante mostrou 68,83% de inibição usando o método de DPPH, demonstrando que a chia pode ser considerada uma semente com alta capacidade antioxidante (Martínez-Cruz & Paredes-López, 2014). Em um estudo, comparou sementes de chia de duas regiões diferentes, Jalisco e Sinaloa, e identificou em maiores concentrações os compostos fenólicos quercetina e o kaempferol, enquanto os ácidos cafeico e clorogênico estavam presentes em menores concentrações (Reyes-Caudillo et al., 2008).

As vitaminas e os minerais são essenciais na nossa alimentação, pois auxiliam no desempenho do organismo como regulação hormonal, diferenciação celular e tecidual, além de atuar como antioxidantes (Pal & Raj, 2020). Um estudo conduzido por Marcinek & Krejpcio (2017), os minerais encontrados nas sementes de chia em maior quantidade foram o fósforo, potássio, cálcio e magnésio. As sementes de chia são fonte de vitaminas como tiamina, riboflavina, niacina e ácido fólico (Marcinek & Krejpcio, 2017). Em um outro estudo com chia

cultivada no Brasil, nos estados de Mato Grosso e Rio Grande do Sul, constatou-se que as sementes de chia tiveram um teor elevado de ferro, zinco, cálcio, manganês, potássio e fósforo (Silva et al., 2016). Em relação a quantidade de vitamina E, concluiu-se que a quantidade observada na chia foi superior ao encontrado em cereais como trigo, aveia, cevada, centeio e sorgo, sendo que essas diferenças podem ser atribuídas a localização geográfica, tipo de solo, clima, umidade e condições de cultivo (Silva et al., 2016).

2.2 **Processo de germinação da chia**

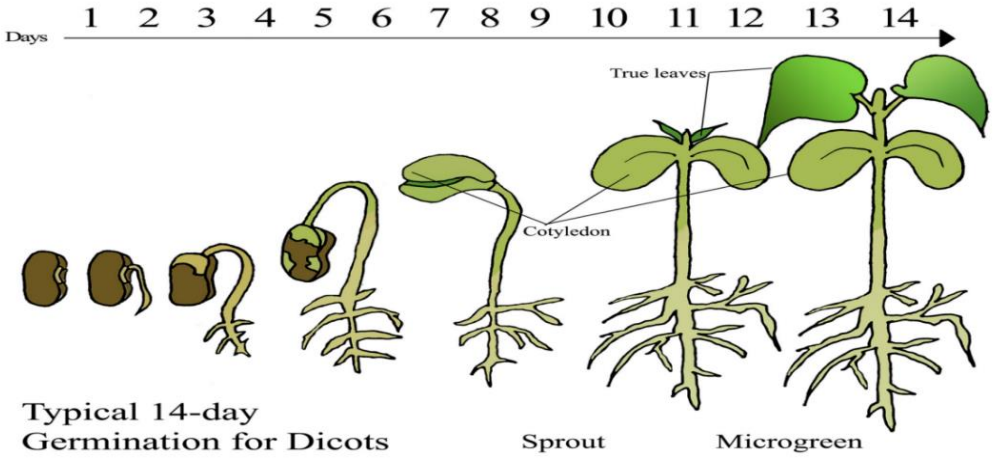
Brotos (*Sprouts*) são o produto obtido a partir da germinação das sementes e do seu desenvolvimento em água ou outro meio, colhido antes do desenvolvimento das folhas verdadeiras e que se destina para ser ingerido inteiro, incluindo a semente (ESSA, 2016). A germinação está associada ao primeiro estágio pós-germinação, nesse período, entre dois e três dias, é que ocorre o desenvolvimento do embrião para originar uma plântula, e é caracterizado por intensa atividade metabólica e pelo consumo de macronutrientes para posterior síntese de novos nutrientes usados para a nova planta (Lourenço & Melo, 2020; Loures et al., 2009; Stefanello et al., 2015). Entretanto, os brotos correspondem a estágios de desenvolvimento mais avançado, com a planta apresentando raiz, haste e clorofila, sem a casca, com altura de 8 a 10 cm e folhas definidas, como mostra na figura 1 (Lourenço & Melo, 2020; Loures et al., 2009).

Germinação é um método barato e simples, não necessita adubos, o cultivo pode ocorrer em pequenos espaços, até mesmo no ambiente doméstico e em condições climáticas adversas (Geng et al., 2022; Miyahira & Antunes, 2021; Warriner, 2014;). Embora a possa acontecer em diferentes condições climáticas, os fatores ambientais influenciam a germinação, como a temperatura, salinidade, luz e umidade do solo (Cabrera-Santos et al., 2021; Nonogaki, 2017; Seo et al., 2009). Em um trabalho realizado por Sorana et al., (2019) , avaliaram-se os efeitos da temperatura, substrato e luminosidade em sementes de chia com o objetivo de determinar condições adequadas para a germinação. Os autores concluíram que o índice de velocidade de germinação foi maior em temperaturas entre 25 e 30 °C, tanto na presença quanto na ausência de luminosidade. Foi observado também que o menor índice de germinação foi alcançado a 35 °C, sugerindo que altas temperaturas reduzem a germinação das sementes de chia. Segundo Stefanello et al. (2015), a eficiência de germinação das sementes de chia não sofre interferência da presença ou ausência de luz, porém em relação à temperatura, a germinação acontece melhor

em temperatura de 20°C. Os autores observaram ainda que outro fator que pode interferir na germinação é a submissão a diferentes níveis de salinidade, que causa uma redução da germinação e no vigor das sementes, classificando a espécie como moderadamente tolerante à salinidade (Stefanello et al., 2020).

A intensidade, qualidade e duração da luz afetam significativamente o crescimento e desenvolvimento das plantas através da morfogênese, da função do aparelho fotossintético e das vias metabólicas (Mlinarić et al., 2020; Wang et al., 2017). Em relação aos trabalhos encontrados, não há um consenso sobre a exposição de luz durante o processo de germinação de semente de chia, uma vez que alguns autores adotaram 12 horas na luz e no escuro, enquanto outros realizaram somente no escuro ou em outra proporção de luz/escuridão (Abdel-Aty et al., 2021; Beltrán-Orozco et al., 2020; Gómez-Favela et al., 2017; Pająk et al., 2019).

Figura 1 - Evolução do desenvolvimento do broto e microgreen



Legenda: Evolução da germinação de 14 dias de feijão comum.
Fonte: Riggio et al., 2019

2.2.1 Composição nutricional e funcional da semente de chia germinada

Um estudo mostrou que o processo de germinação foi capaz de aumentar o conteúdo de proteínas em 13% durante as 48 horas iniciais e que após esse tempo houve uma diminuição (Beltrán-Orozco et al., 2020). O subsequente declínio de proteínas após 2 dias de germinação pode ser devido ao seu uso para a obtenção de energia ou para a síntese de outros componentes necessários ao crescimento (Beltrán-Orozco et al., 2020). Gómez-Favela et al., (2017) encontraram um aumento de 20,9% no teor de proteína após 156 h de germinação a 21 ° C.

De acordo com Beltrán-Orozco et al. (2020), a germinação induz um aumento na proporção de aminoácidos essenciais encontrando, em seu estudo, um aumento de aproximadamente 100% no teor de triptofano após 4 dias. No estudo de Gómez-Favela et al., (2017), os autores observaram que o teor de aminoácidos essenciais das sementes de chia aumentou significativamente após o processo de germinação, bem como um aumento de 4,8% na digestibilidade da proteína *in vitro*. Esse comportamento difere dos observados por outro estudo, pois a digestibilidade *in vitro* da proteína diminui à medida que a germinação progride, justificada devido ao aumento paralelo de fibras e compostos fenólicos (Beltrán-Orozco et al., 2020).

Existem poucos estudos sobre perfil de ácidos graxos de sementes de chia germinadas. Apenas em um estudo, foram investigadas as alterações no perfil de ácidos graxos durante a hidratação de sementes de chia a 10°C, 20°C e 30°C, para determinar a correlação entre os ácidos graxos, a temperatura e a germinação. Os resultados mostraram que as maiores concentrações dos ácidos palmítico, esteárico, linoleico e linolênico foram observadas na temperatura de hidratação de 20°C (Cabrera-Santos et al., 2021). Isso indica a necessidade de mais estudos voltados ao perfil de ácidos graxos na semente de chia após o processo de germinação.

Beltrán-Orozco et al. (2020) mostraram que o teor total de fibra alimentar aumentou em aproximadamente 46% após 4 dias de germinação da semente de chia. Já para o autor Gómez-Favela et al., (2017), o teor total de fibra alimentar das sementes de chia após a germinação (GT = 21 °C/ Gt = 157 h) aumentou 3,39%, sendo que a fibra insolúvel aumentou 5,14% e a fibra alimentar solúvel diminuiu 13,53%. Segundo os autores, esse efeito pode ser devido à perda de mucilagem quando as sementes entram em contato com a solução de hidratação no início do processo de germinação.

Abdel-Aty et al., (2021) avaliaram o impacto do processo de germinação da semente de chia sobre o perfil de compostos fenólicos, propriedades antioxidantes e antibacterianas de sementes de chia germinada. Os autores observaram que os teores totais de compostos fenólicos, dentre eles os flavonóides, tiveram um aumento até o sétimo dia de germinação de 6,4 e 11,5 vezes, respectivamente. Posteriormente, os autores observaram que ocorreu uma diminuição no conteúdo de fenólicos totais, principalmente flavonoides. Isso pode ter ocorrido devido à conversão de alguns compostos fenólicos livres em compostos fenólicos ligados ou consumidos para síntese de parede celular. Nesse mesmo estudo, a atividade antibacteriana das sementes de chia germinada foi melhorada/aumentada após o processo de germinação e os

autores sugeriram ter sido devido ao aumento nas concentrações dos compostos fenólicos identificados devido a produção de novos compostos (Abdel-Aty et al., 2021).

Gómez-Favela et al., (2017), produziram farinha de chia germinada e encontraram o conteúdo fenólico total de 3,38 mg GAE/g após 155 h de germinação a 21° C. Já em outro estudo, o valor do conteúdo total de fenólicos totais aumentou 3 vezes após o quarto dia de germinação (Beltrán-Orozco et al., 2020). Os resultados são semelhantes aos encontrados por outros pesquisadores em que a chia germinada teve um aumento de 0,92 a 4,40 mg GAE g-1 comparado a semente de chia não germinada (Pająk et al., 2019).

Dentre os compostos fenólicos predominantes do broto de chia, podemos citar uma série de compostos identificados ácido rosmarínico, ácido p-cumárico, ácido cafeico, entre outros (Abdel-Aty et al., 2021). Esses compostos são reconhecidos por suas propriedades antioxidantes, anti-inflamatórias e antimicrobianas, que desempenham um papel crucial na proteção contra danos oxidativos, na modulação da resposta inflamatória e na promoção da saúde cardiovascular (Abdel-Aty et al., 2021).

Um estudo de Motyka et al. (2023), ao analisar o perfil de compostos fenólicos do broto de chia, foram identificados um total de 19 compostos, entre eles, o ácido rosmarínico foi o composto que apresentou a maior concentração. Esses resultados destacam a importância do ácido rosmarínico como um componente significativo dentro da composição dos compostos fenólicos analisados, a presença predominante desse composto sugere que ele pode desempenhar um papel crucial na capacidade antioxidantes e atividades biológicas observadas.

O ácido rosmarínico é um composto fenólico, formado a partir dos ácidos cafeico e 3,4-di-hidroxifenillatico, comumente encontrado em extrato de diversas espécies de plantas, principalmente da família Lamiaceae (Hitl et al., 2021). O composto demonstra uma ampla gama de atividades biológicas, abrangendo propriedades antissépticas, antioxidantes, antiinflamatórias, antivirais, hipoglicêmicas, antitumorais e neuroprotetoras (Guan et al., 2022). A sua ampla variedade de propriedades biológicas tem levado a investigações científicas para compreender melhor os seus efeitos e potenciais aplicações terapêuticas em brotos (Abdel-Aty et al., 2021; Hunaefi & Smetanska, 2013; Motyka et al., 2023)

Além disso, o tempo de germinação dos brotos teve um efeito positivo na atividade antioxidante, sugerindo que os brotos de chia podem neutralizar diferentes tipos de radicais livres e inibir a formação de radicais livres mediada por metais (Abdel-Aty et al., 2021; Beltrán-Orozco et al., 2020; Gómez-Favela et al., 2017). Estudo relatam a evidência de que os brotos de chia podem ter uma atividade antioxidante superior às sementes, o que pode desempenhar

um papel importante na prevenção de danos causados pelos radicais livres e no combate ao envelhecimento celular e ao desenvolvimento de doenças crônicas (Grancieri et al., 2019).

2.3 **Aspectos microbiológico da germinação**

Apesar dos alimentos germinados serem reconhecidos por seu alto valor nutricional, é importante ressaltar que eles vêm sendo associados a surtos de doenças transmitidas por alimentos. São totalizados 14.739 casos de surtos no mundo, no período de 1988 a 2020, dentre eles o broto de alfafa estava envolvido em mais da metade dos casos (Miyahira & Antunes, 2021). Um surto internacional ocorreu, nos EUA e Canadá, onde cerca de 96 pessoas foram infectadas com cepas de *Salmonella*, entre os anos de 2013 e 2014, ligadas a farinha de sementes de chia germinadas (Harvey et al., 2017). Outro exemplo citado por Symes et al., (2015), entre os anos 2005 e 2006, no país da Austrália, mais de 120 casos de salmonelose relacionados ao consumo de brotos crus foram registrados. Segundo o autor Loures et al., (2009), sua pesquisa avaliou o perfil microbiológico de broto de lentilha PRECOZ, seus resultados mostraram que o valor para coliformes totais e coliformes fecais foi superior, indicando possível contaminação que pode ser proveniente da própria semente ou contaminação durante a manipulação e preparo dos brotos ou da água utilizada. Esses exemplos evidenciam a importância de garantir boas práticas de higiene e segurança alimentar ao consumir alimentos germinados, a fim de minimizar o risco de doenças transmitidas por alimentos associadas a esses produtos.

A *U.S. Food and Drug Administration* (FDA), juntamente com o Instituto de Segurança e Saúde Alimentar no Instituto de Tecnologia de Illinois criou a *Sprout Safety Alliance* (SSA), para dar suporte aos produtores de brotos a identificar e implementar a produção segura de brotos, pois apresentam risco de segurança alimentar (*Sprout Safety Alliance | FDA*, n.d.).

Geralmente, os brotos são ingeridos sem nenhum tipo de tratamento para reduzir a carga de microrganismos, além disso, as condições em que ocorre a germinação são propícias para a proliferação de microrganismos que possam estar presentes nas sementes (Harvey et al., 2017).

É necessário destacar que a contaminação pode ocorrer em diversas etapas durante a produção das sementes, incluindo a pré-colheita, em que existe a possibilidade de internalização de patógenos através da rizosfera pelo sistema vascular da planta, representando uma possível via de contaminação de sementes (Warriner, 2014).

Na pré-colheita, os procedimentos para evitar a contaminação de sementes estão relacionados à qualidade das mesmas e aos procedimentos de higiene da lavoura (Miyahira & Antunes, 2021). Existem estratégias para minimizar esse risco de contaminação como controlar a qualidade da água de irrigação, além da saúde e higiene dos manipuladores (Miyahira & Antunes, 2021).

Na pós-colheita, o objetivo é diminuir a carga microbiana da semente/grão que foi obtido no campo de plantação, e para isso é recomendado que se utilize dois métodos de sanitização para garantir a redução de patógenos. O método químico mais comumente usado é o hipoclorito de sódio (Sikin et al., 2013; Warriner, 2014). Outra estratégia é a triagem das sementes, com o objetivo de identificar lotes contaminados e cessar essa distribuição (Warriner, 2014). Os métodos físicos são alternativas aos métodos químicos, sendo possíveis o uso de calor, irradiação, uso de alta pressão, luz ultravioleta e plasma frio atmosférico (Miyahira & Antunes, 2021; Sikin et al., 2013).

Durante o processo de germinação, boas práticas de manejo precisam ser implementadas, como o uso de água tratada e filtrada para a irrigação das sementes e o uso de recipientes limpos (Miyahira & Antunes, 2021). Após a germinação, é importante o armazenamento correto dos brotos sob a refrigeração para evitar o crescimento de bactérias patogênicas nessa fase, além do transporte e condições ambientais adequadas (Miyahira & Antunes, 2021; Warriner, 2014).

3 **JUSTIFICATIVA**

A semente de chia é utilizada pela população devido a suas propriedades benéficas à saúde, tais como aumento da saciedade, regularização da função intestinal, diminuição do risco de doenças crônicas não transmissíveis. A germinação da chia parece aumentar essas propriedades e ainda ser capaz de diminuir alguns fatores antinutricionais. Entretanto, ainda existem poucos estudos na literatura científica que avaliaram as mudanças que ocorrem na semente de chia após a germinação em diferentes tempos, especialmente relacionados ao perfil de ácidos graxos.

Além disso, devido ao elevado valor nutricional e às condições de germinação, estas sementes podem propiciar o desenvolvimento de microrganismos patogênicos. Sendo assim, conhecer quais os microrganismos estão sendo isolados de brotos e se os meios de sanitização são capazes de reduzir esses microrganismos, torna-se necessário para posterior desenvolvimento de ações que possam subsidiar regulamentações que garantam a produção segura de brotos, uma vez que estes estão cada vez mais sendo cultivados em ambientes domésticos e usados em saladas ou outros pratos que não sofrem nenhum tratamento térmico para redução de patógenos.

4 **OBJETIVOS**

4.1 **Objetivo geral**

Avaliar a qualidade nutricional e microbiológica de sementes de chia (*Salvia hispânica* L.) durante o processo de germinação.

4.2 **Objetivos específicos**

- Analisar a composição centesimal da semente de chia antes e depois da germinação;
- Determinar e identificar o conteúdo de compostos fenólicos totais da semente de chia antes e depois da germinação;
- Determinar a atividade antioxidante da semente de chia antes e depois da germinação;
- Analisar o perfil de ácidos graxos da semente de chia antes e depois da germinação;
- Determinar a quantidade de coliformes totais, *Escherichia coli*, bactérias aeróbias mesófilas e *Bacillus cereus* em brotos de chia submetidos à processo de sanitização;
- Avaliar a presença de *Salmonella* spp. e *Listeria monocytogenes* em brotos de chia submetidos à processo de sanitização.

5 CAPÍTULO 1

Chia (*Salvia hispanica* L.) seed germination: a brief review

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ABSTRACT

Chia (*Salvia hispanica* L.) is a seed native to northern Mexico and southern Guatemala that has started to be consumed in recent years in other regions of the world owing to its nutritional and functional properties. Germination of chia seeds seems to be able to further improve these properties, and it has been the subject of some studies. In general, germination has proven to be a simple and inexpensive process capable of improving the content of phenolic compounds and the antioxidant capacity of foods, as well as reducing antinutritional factors that interfere with nutrient absorption. A particular characteristic of chia seeds is that they produce mucilage when they are hydrated. For this reason, the germination conditions of the seed need to be adapted. The nutritional guidelines of some countries, such as Brazil, Germany and Sweden, recommend that the diet of the population should be more plant-based, thus encouraging the consumption of foods with a high content of bioactive compounds and nutrients, e.g., germinated seeds. This review briefly explored the germination conditions of chia seeds as well as the changes in phytonutrient content and antinutritional factors after their germination process. The main information available in the literature is that germination of chia seeds can increase the contents of protein, fiber, and total phenolic compounds. As a conclusion, germination of chia seeds is favorable for increasing their health benefits and nutritional value. However, chia germination parameters should be adjusted and microbiological risks should be properly evaluated.

Keywords: Germination process, Plant-based diet; Phenolic compounds; Antioxidant activity; Antinutritional factors

Introduction

Salvia hispanica L., popularly known as chia, is an ancient herbaceous plant of the Lamiaceae family, native to Southern Mexico and Northern Guatemala [1, 2], which has recently been commercialized as a crop in South America [3]. Chia seed was recognized as a novel food ingredient by the European Parliament in the 1990s [4] owing to its health benefits and has been associated to lower incidence of non-communicable chronic diseases, e.g., cardiovascular disease, obesity, hypertension, diabetes, and cancer [5-7]. The health benefits of chia seed are attributed to its nutrition and functional properties since it is a source of fat, protein, dietary fiber, mineral [8-15]. Table 1 shows data on the nutritional composition of chia found in the scientific literature.

Regarding lipids, chia seeds are considered a food source with a high content of omega-3 and omega-6 fatty acids [16]. A recent scientific report examined sixteen transcriptomes obtained from seeds of eight different cultivated and wild accessions of *S. hispanica*, representative of the biodiversity of this crop [16]. The study found that domestication of chia could be associated with the accumulation of storage lipids in the cultivated accessions and that genes responsible for the biosynthesis and degradation of triacylglycerols and fatty acids showed differences in expression between cultivated and wild accessions. In addition, the study provided evidence that the expression of transcripts related to lipid metabolism in chia seeds may be affected by different growth conditions [16].

Chia seeds are also a rich source of protein, making them a very attractive source for human nutrition and health, with much higher levels than in other crops such as oats, wheat, and rice [16]. In a study to identify the protein fractions of commercial chia varieties, it was found that the concentration of globulins was the highest, followed by the protein fractions albumin, glutelin, and prolamin [17].

Chia seed consumption has also been reported for its high antioxidant potential [7, 18]. Several studies have shown the capacity of chia to scavenge free radicals and reduce oxidative stress [11, 19-21]. Phenolics composition is an important factor that is responsible for the antioxidant activity of chia seeds [22]. Another important characteristic of chia seeds, which is responsible for satiety during intake and for their high potential as a functional ingredient to be added in foods, is the content of mucilage, which forms a thick layer when soaked in water (gel) [23, 24].

Chia can be consumed as whole seeds or in the form of flour, mucilage (gel) and oil, alone (*in natura*), added to other foods (yogurts, fruits, salads, and soups) or as ingredients of preparations (breads, cakes, whole grain bars, and beverages) [25]. Adding chia to foods improves their physicochemical and sensory characteristics as well as increases their nutritional content and health properties [19, 26]. There has been recent research on germinated chia seeds because their germination seems to be able to increase the nutritional and nutraceutical values of foods [27, 28].

Germination is a simple process in which water is added to the seeds until they have developed the embryonic axis. It is a process that does not require soil neither controlled light, temperature, and humidity conditions; in addition, it is inexpensive and can be carried out in a domestic environment [29]. Although simple, germination requires careful monitoring to provide further insights into the changes that occur during the process, with a view to determining the optimal conditions that result in a safe and quality product [30]. Sprout is the advanced stage of seed germination and, according to the European Parliament, it is "the product obtained from the germination of seeds and their development in water or another medium, harvested before the development of true leaves and which is intended to be eaten whole, including the seed" [31, 32].

A wide range of sprouts have been studied for their high nutritional value and therapeutic properties and are increasingly present in the diet of people who seek a close relationship between diet, health, and the environment [33]. Germination is an effective green food development strategy to increase the phytonutrient content in seeds because it reactivates their metabolism, which leads to catabolism as well as macronutrient degradation and stimulates the synthesis of secondary metabolites that have health benefits [30, 34, 35]. Germination is able to induce enzymes to break down carbohydrates, lipids, and proteins in basic methods, and stimulates proteases involved in protein destruction, thus improving nutrient bioavailability [30].

Although there are still few studies, the literature reports that germination of chia seeds results in increased protein content and quality, and increased total phenolic compounds and antioxidant capacity [28]. More recently, Cabrera-Santos et al. [36] explored lipid profile changes during chia seed germination and showed an increase in fatty acid content, depending on the phase and temperature of germination. Chia sprouts can be consumed *in natura* by being added to salads, soups, and sandwiches, or be used to improve the nutritional and functional value of food products [37, 38].

The addition of sprouted chia to food products has not been widely explored in the scientific literature. Argüelles-López et al. [37] developed a functional beverage based on chia and amaranth processed by germination and extrusion, and they evaluated its nutritional, antioxidant and antihypertensive properties. To this end, two functional beverages were developed from flour mixtures: Mixture 1 (70% extruded amaranth flour + 30% sprouted chia flour) and Mixture 2 (70% sprouted amaranth flour + 30% sprouted chia flour). In the sensory evaluation performed with panelists, the beverages had high acceptability with average values of 83-85. The authors concluded that the beverages had a high content of high-quality protein and dietary fiber, with low energy content and high sensory acceptability. In addition, the beverages had high antioxidant and antihypertensive potential and may be a good alternative to beverages with high calories and low nutritional value, which predominate in a consumer market with significant trends towards overweight and chronic degenerative diseases [37].

The use of germinated chia as a cooking ingredient can be a good strategy to increase the nutritional value of food products. Moreover, the addition of chia sprouts in culinary preparations can reduce the risk of foodborne diseases, since the consumption of sprouts, which are usually raw, is associated with several foodborne outbreaks, and cooking them is able to reduce their microbial load [39]. Harvey et al. [38] reported an international outbreak of multiple *Salmonella* serotype infections in 2013-2014, linked to sprouted chia seed powder. Ninety-four people were found to be infected with outbreak strains from 16 states, in which 21% were hospitalized. The authors reported that although chia seed powder is a new outbreak vehicle, sprouted seeds are recognized as an important cause of foodborne illnesses [38].

In addition, it should be noted that the promotion of habitual consumption of chia sprout may be an important action in the context of plant-based diets, which are well- recognized for provide physical and environmental health benefits [33, 40]. Considering that chia sprouts have been increasingly studied for their nutritional and functional potential, this review explores the germination conditions of chia seeds, and the changes in the content of phytonutrients and antinutritional factors after germination. The literature search was carried out using scientific databases comprising Scopus, Web of Science, PubMed, using the following keywords: “chia”, “chia sprout”, “*Salvia hispanica* L.”, “sprout”, “germination” and “seeds”. The articles found about chia sprouts were published between 2017 and 2021.

Optimizing the Chia Germination Process

Germination is often considered to be a critical stage in the life cycle of plants, as it is highly sensitive to environmental factors such as temperature, light, water availability, and gaseous environment [36, 41, 42]. It is worth noting that chia seeds release mucilage during the imbibition phase [36]. It has been suggested that mucilage inhibits germination under excessively humid conditions by preventing the diffusion of oxygen to the embryo [43]. To date, few studies in the scientific literature have shown the optimal conditions for sanitization process, light and temperature [36, 44]. Most studies have established germination conditions based on preliminary tests in the laboratory and adopted the strategy of using germination paper and spraying water on the seeds on a daily basis [27, 28, 45]. Table 2 shows the objectives, germination conditions, and the main results found in the studies with germinated chia. It should be noted that the germination conditions were different, as well as the origin of the chia seed in some studies.

Sanitization

As a pre-germination step of any seeds, measures should be adopted to decrease their microbial load, such as sanitizing the seeds prior to the germination process, since germination presents optimal conditions for microbial development [39]. In a study conducted by Abdel-Aty et al. [28], chia seeds were sanitized with sodium hypochlorite, while Beltran-Orozco et al. [45] previously hydrated chia seeds and then washed them with liquid detergent, whereas ethanol was used to sanitize chia seeds in another study [27].

Light

Light intensity, quality, and duration significantly affect plant growth and development through morphogenesis, photosynthetic apparatus function, and metabolic pathways [44, 46]. Mlinari et al. [44] suggested that lighting has a positive effect on the antioxidant potential of chia seeds, as light conditions can enhance the synthesis of different antioxidants. In the studies found in the scientific literature, there is no consensus on light exposure during the chia germination process, with some studies adopting 12/12 hours in light and dark [27, 47], while others performed germination only in the dark [28, 45]; still, others used different light/dark ratio [48].

Temperature

Temperature is the main controlling factor in germination and its effect has been related to the seeds' water uptake, level of latency, rate of seed deterioration, and length of time during which germination takes place [36, 42]. As with the other parameters mentioned above, there is no consensus on the ideal temperature for chia germination. Abdel-Aty et al. [28] germinated chia seeds at room temperature (25-30°C). These values were close to those described by Gomez-Favela et al. [47] (20-35°C) and by Beltran-Orozco et al. [45] (up to 30°C). Lower temperatures were used by Stefanello et al. [48] and Pajak et al. [27]: 20°C and 22±2°C, respectively.

One study determined the best combination of process variables for producing optimized sprouted chia flour and found the optimal combinations of bioprocess variables (21 °C/157 h; 33 °C/126 h) to produce two flours with higher protein, lipid, phenolic compound and antioxidant activity contents. Only the flour made with chia germinated at 21 °C for 157 h was considered as adequate, because the samples germinated at 32 °C for 126 h tended to have fungal growth and a small number of germinated seeds [47].

Nutritional value of germinated chia

In general, almost all nutrients in sprouted grains are more available, and several antioxidants occur in higher concentrations; for this reason, sprouts can be considered as "functional foods" [49]. Some changes in phenolic compounds and nutritional composition were found in germinated chia (Fig 1). Table 3 shows data on the nutritional composition of chia sprouts found in the scientific literature. These changes will be reported in more detail below.

Phenolic compounds

Studies have shown that dietary intake of bioactive components, such as phenolic compounds in chia seeds, is associated with reduced risk of cardiovascular disease and hepatoprotective effects, as well as protection against plasma oxidative stress and obesity-related diseases [50, 51]. Abdel-Aty et al. [28] evaluated the impact of germination on the antioxidant and antibacterial properties of sprouted chia seeds. The results showed that total phenolic and flavonoid contents had a 6.4- and 11.5-fold increase until day 7 of chia seed

germination, respectively. After this germination period until day 10, there was a decrease in total phenolic and flavonoid contents, which may have been due to the conversion of some free phenolic compounds to bound phenolic compounds or to the ones used for cell wall synthesis. Furthermore, the results also suggested that the antibacterial activity of chia seeds was enhanced after germination, which may have been due to an increase in the concentrations of all identified phenolic compounds or to the production of new ones [28].

Gomez-Favela et al. [47] produced germinated chia flour and found a total phenolic content of 3.38 mg GAE/g after 155 h of seed germination at 21° C. In another study, the value of total phenolic content increased 3-fold after the fourth day of germination. Moreover, 30% of the total phenolic content was represented by total flavonoid content and its content increased 197% after 4 days of germination [45]. The results were similar to those of Pajak et al. [27], who found an increase in phenolic compounds from 0.92 to 4.40 mg GAE g-1 in germinated chia when compared to ungerminated chia seeds.

Table 3 shows that the values found for total phenolic compounds in the studies were different from each other. This may have occurred because of the different germination conditions and origins of the study seeds [28].

Protein content

The protein content of chia seeds is about 17%, higher than the protein content in all other cereals [2]. According to Beltran-Orozco et al. [45], the germination process increased the protein content by 13% during the initial 48 hours of the germination process, but there was a decrease after that time. This subsequent decline in protein after 2 days of germination may have been due to its use for energy or for the synthesis of other components required for plant growth. Furthermore, germination induced an increase in the proportion of essential amino acids and resulted in an approximately 100% increase in tryptophan content after 4 days of chia seed germination [45].

Another study found a 20.9% increase in protein content after 156 h of germination at 21 °C [47]. In addition, the essential amino acid content of chia seeds increased significantly after this germination process, and there was also a 4.8% increase in protein digestibility in vitro [47]. This behavior differs from those found by Beltran-Orozco et al. [45], since in vitro protein digestibility decreased as germination progressed, possibly owing to the parallel increase in fiber and phenolic compounds.

Gamma-aminobutyric acid (GABA)

In a study using germinated chia seed flour, GABA content in chia seeds was evaluated before and after the germination process. The results showed that GABA content in chia seeds was 9.51 mg/100 g dry weight and 117.66 mg before and after the germination process, respectively [47]. Therefore, the germination of chia seeds showed a significant ($p < 0.05$) 11.4-fold increase in GABA content. This increase may have been due to decarboxylation of L-glutamic acid and catalyzation by glutamate decarboxylase (GAD) during seed germination. This is a positive result, because GABA has antidiabetic, anti-hypercholesterolemia, antihypertensive, anti-inflammatory and antidepressant properties, and antiproliferative effects against cancer cells [52].

Dietary fiber content

Dietary fiber intake can reduce the risk of diseases such as diabetes, obesity, coronary heart disease, hypertension, stroke, and gastrointestinal disorders [53]. Total fiber content increased by approximately 46% after 4 days of chia seed germination [45]. In another study, the total dietary fiber content of chia seeds after germination (157h) increased by 3.39%, with insoluble fiber increasing by 5.14% and soluble dietary fiber decreasing by 13.53%. According to the authors, this effect may have been due to the loss of mucilage when the seeds come into contact with the hydration solution early in the germination process [47]. It is worth noting that the values found for dietary fiber were very different between these studies, since one analyzed total dietary fiber while the other, crude fiber (Table 3).

Lipid content and fatty acid profiles

Chia seeds stand out for their lipid profile, showing approximately 25-40% of their total weight in lipids, with 50-57% being linolenic acids and 17-26% being linoleic acids [36]. The lipid content of chia seed meal after germination at 21 °C for 157 h had a significant ($p < 0.05$) decline from 33.7 ± 0.16 to 15.06 ± 0.60g/100g dw [47]. Another study found an increase in the amount of lipids after chia germination for 48h and a significant reduction after germination

for 96h compared to ungerminated chia (Table 3) [45]. The reduction in seed oil content during germination can be attributed to the use of energy for metabolic activity, such as synthesis of DNA, RNA, enzymes, structural proteins, and other [54].

Regarding lipid profile, a study investigated the changes in fatty acids during hydration of chia seeds at 10°C, 20°C and 30°C to determine the correlation between fatty acids, temperature and germination. The results showed that the highest concentrations of palmitic, stearic, linoleic and linolenic acids were found at 20°C [36]. It should be noted that no studies were found in the scientific literature that evaluated changes in the profile of fatty acids after chia germination.

Antinutritional factors *versus* mineral and vitamin contents

There is evidence to indicate that germinated foods are superior in nutrients, such as minerals, compared to their ungerminated counterparts owing to the activation of endogenous enzymes that degrade the antinutritional factor, e.g., trypsin inhibitors in legumes, tannins in legumes and cereals and phytates in cereals [55, 56]. Antinutritional factors may occur naturally in plants as part of their protection against attacks by herbivores, insects, and pathogens or as a means of survival under adverse growing conditions [57]. The presence of antinutritional factors in food can restrict the digestion of proteins and bioavailability of different minerals [58]. For better understanding, the terms bioaccessibility and bioavailability should be distinguished. Bioaccessibility is the fraction of compounds that is released from the food matrix during digestion and becomes available for absorption in the intestine, while bioavailability refers to the fraction of compounds that is absorbed, distributed through the circulatory system, and subject to metabolism and elimination [59].

During germination, there is a frequent increase in mineral content as a result of the hydrolysis of phytic acid, owing to an increase in phytate enzymatic activity [27]. Phytic acid is an antinutritional factor that acts as a powerful chelating agent, thus reducing the bioavailability of minerals that form insoluble complexes [27].

Few studies have aimed to verify the content of minerals in chia before and after germination. Pajak et al. [27] evaluated the content of sodium, potassium, calcium, magnesium, iron, manganese, copper, and zinc in chia seeds and in 7-day germinated chia. The results

showed that there was a significant increase in the amount of calcium and iron in germinated chia compared to ungerminated chia seeds [27].

This is in agreement with the study by Calvo-Lerma et al. [60], who also analyzed the calcium content in chia seeds and in germinated chia. The results showed that there was a significant increase in calcium content in the 10-day germinated chia sample [60].

Another study evaluated the ascorbic acid content in chia seeds and in 4-day germinated chia. The authors found that the ungerminated chia seeds had no detectable ascorbic acid, but germination produced an increase of this vitamin as of the second day and continued to increase significantly over the next 2 days [45]. This increase is related to the activity of GLDH (L-galactono-clactone dehydrogenase), a key enzyme in ascorbic acid biosynthesis, which increases significantly during germination, reactivating vitamin C biosynthesis [61].

It is worth noting that the studies mentioned above did not evaluate the reduction of antinutritional factors, but only compared nutrient content before and after chia seed germination. Considering the lack of studies on this topic, it is expected that future studies will be developed to evaluate the behavior of the antinutritional factors at different germination times of chia seeds.

Conclusions and perspectives

Germinating chia seeds can increase their health benefits and nutritional value. However, the seeds' own mucilage can inhibit the germination process. Therefore, the germination parameters of chia need to be adjusted. Few studies were found in the scientific literature that evaluated the optimal germination conditions of chia seeds at different periods, as well as the impact of germination on the nutritional value of these seeds. More studies are needed to evaluate the reduction of antinutritional factors in germinated chia seeds and the consequent increased bioavailability of nutrients, as well as to evaluate the fatty acid profile of chia during germination. In addition, in vivo and in vitro studies should be conducted to verify the benefits of consuming sprouted chia, as well as if there is a chance of toxicity.

The consumption of a more plant-based diet is increasing worldwide, and sprouted seeds may be a promising dietary option, and they may thus favor a higher intake of phenolic compounds, minerals, gamma-aminobutyric acid (GABA), fiber, omega-3 fatty acid, and others.

Declarations

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Availability of data and material

Data is available upon request.

Authors' contributions

R.M. had the idea for the article. V.S. performed the literature search and data analysis. R.M., L.Z., V.S. and A.A. wrote the main manuscript text. V.S. prepared Table 2 and Figure 1. R.M. prepared Tables 1 and 3. All authors drafted and/or critically revised the work.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Statement

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

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Table 1 – Centesimal composition and total phenolic content of chia seeds (ungerminated) observed in the scientific literature

Component	*Hernández-Pérez et al.* [17]*	*Marineli et al.* [50]*	*Segura-Campos et al. [11]*	*Fernandes et al.* [12]*	*Monroy-Torres et al. [13]*	*Beltran-Orozco et al. [45]*	*Gómez-Favela et al.*, ** [47]*
Moisture (g/100g)	4.50 ± 0.00	5.82 ± 0.04	6.32	5.74 ± 0.17	6.25		
Lipids (g/100g)	32.50 ± 2.70	30.22 ± 0.08	34.88	35.68 ± 0.61	33.00	37.98 ± 2.79	33.70 ± 0.16
Protein (g/100g)	22.70 ± 0.70	25.32 ± 0.21	23.99	19.55 ± 0.25	18.65	20.66 ± 0.10	18.48 ± 0.76
Ash (g/100g)	3.70 ± 0.30	4.07 ± 0.02	4.32	4.93 ± 0.03	4.35	5.06 ± 0.02	3.20 ± 0.30
Total Dietary fiber (g/100g)	33.50 ± 2.70	37.50 ± 1.07	35.85	17.18 ± 0.04****	28.38	16.60 ± 0.36****	42.52 ± 0.43
Soluble Dietary fiber (g/100g)	8.20 ± 0.80	2.43 ± 0.30	-	-	-	-	3.99 ± 0.043
Insoluble Dietary fiber (g/100g)	25.40 ± 2.20	35.07 ± 0.90	-	-	-	-	38.53 ± 0.47
Carbohydrates (g/100g)	3.10	34.57 ± 0.26	-	22.66	9.37	-	44.62 ± 0.4
Total phenolic content (mg GAE/g)	0.78 ± 0.04 - 0.97± 0.03***	0.94 ± 0.06	-	-	-	0.98 ± 0.03	-

- Analysis not performed

*Values are the mean ± standard deviation

** Ungerminated chia flour

*** Range of four varieties of commercial chia seeds

**** Crude fiber determination

Table 2 - Summary of studies conducted with germinated chia seeds reported in the scientific literature

Reference	Objective	Origin of chia	Germination conditions					Main results
			Sanitization	Place	Temperature	Time	Light	
Abdel-Aty et al. (2021) [28]	To evaluate the impact of the germination process on the phenolic profile, antioxidant, and the enzymatic activities.	Egypt	0.07% sodium hypochlorite solution at room temperature for 5 min	Plastic tray containing moistened tissue paper	Room temperature (25-30 C)	1 to 10 days	Dark	↑ Total phenolic and flavonoid contents of chia seeds until 7-day sprouts ↓ total phenolic content between day 7 and 10 Detection of 12 phenolic acids and 5 flavonoids ↑ total antioxidant activity

Beltrán-Orozco et al.(2020) [45]	To evaluate the effect of germination on protein, fat, fiber, ash, tryptophan, vitamin C, total phenolic compounds and total flavonoids, protein digestibility	Mexico	The seeds were placed in water for 10 min for hydration and then washed with liquid detergent.	Plastic trays containing filter paper sheet	Up to 30°C	0, 1, 2, 3 and 4 days	Dark	**Germination for 2 days:** ↑ protein content **Germination for 4 days** ↑ contents of fiber, tryptophan, total phenolics and flavonoids ↑ Vitamin C ↑ Antioxidant capacity ↓ protein digestibility

	and antioxidant activity						
Gomez-Favela et al. (2017) [47]	To investigate the antioxidant activity, phenolic compounds, GABA, essential amino acids, and dietary fiber of	Mexico	-	Placed on plates with absorbent paper previously moistened with sodium hypochlorite solution and placed in a germination chamber	21 °C	57 h	12/12h light and dark	↑ protein content ↑ total dietary fiber content ↑ insoluble fiber ↓ soluble dietary fiber by

	germinated chia seeds.							
Pajak et al. (2018) [27]	To investigate the effect of germination on antioxidant properties, phenolic compounds, and mineral composition.	Austria	Ethanol 96%	Spread on sterile stackable trays and sprayed twice a day	22 ± 2°C	7 days	12/12h light and dark.	↑phenolic compounds ↑ antioxidant activity. > amount of caffeic acid

Stefanello et al. (2020) [48]	To evaluate the influence of different salts on the germination of chia seeds.	Argentina	-	Placed on plastic boxes and kept in chambers	20°C	7 and 14 days	8/16h light and dark.	↓ germination in the presence of salts (NaCl, KCl, CaCl2 and MgCl2)
Cabrera-Santos et al. (2021) [36]	To explore fatty acid changes during chia seed imbibition, to establish	Mexico	-	Placed inside woven mesh cotton bags, in Petri dishes and kept inside germination chambers	10°C, 20°C, and 30 ◦C	1 to 14 days	12/12h light and dark.	↑germination rates at 30 ◦C ↑ fatty acid concentration after 3 h of imbibition ↓ correlation between linoleic and linolenic acid at 20 ◦C

	a correlation between fatty acid behavior, temperature, and germination	25 seeds were sown on agar medium in Petri dishes.	Formation of three isomers of trans linolenic acid at 30 ◦C

Table 3 – Centesimal composition and total phenolic content of chia sprouts found in the scientific literature

Component	*Abdel-Aty et al.* [28]*			*Beltran-Orozco et al.* [45]*				*Gómez-Favela et al.*, ** [47]*	*Pająk et al. [27]*
	Time of germination (day)								
	1	7	10	1	2	3	4	6.5	7
Lipids (g/100g)	-	-	-	41.65 ± 0.88^{c}	42.16 ± 0.31^{d}	38.44 ± 0.79^{b}	30.98 ± 0.04^{a}	15.06 ± 0.60	-
Protein (g/100g)	-	-	-	22.10 ± 0.47^{a}	23.24 ± 0.07^{b}	22.16 ± 0.10^{a}	21.24 ± 0.02^{a}	22.34 ± 0.51	-
Ash (g/100g)	-	-	-	5.01 ± 0.01^{a}	5.02 ± 0.03^{a}	5.04 ± 0.04^{a}	5.12 ± 0.04^{a}	5.10 ± 0.20	-
Total Dietary fiber (g/100g)	-	-	-	18.66 ± 0.02^{a} ***	21.14 ± 0.52^{b} ***	22.30 ± 0.70b^{c} ***	24.25 ± 0.09^{c} ***	43.96 ± 0.05	-
Carbohydrates (g/100g)	-	-	-	-	-	-	-	57.5 ± 0.60	-
Total phenolic content (mg GAE/100g)	430.00 ± 21^{a}	900.00± 42^{b}	680.00 ± 29^{c}	148.60 ± 0.70^{a}	165.40 ± 7.33^{b}	191.10 ± 3.50^{c}	293.60 ± 1.30^{d}	612.00 ± 7.90	440.00

Different letters in the same row mean significant differences in the study.

- Analysis not performed

*Values are expressed as mean ± standard deviation

** Ungerminated chia flour

*** Crude fiber determination

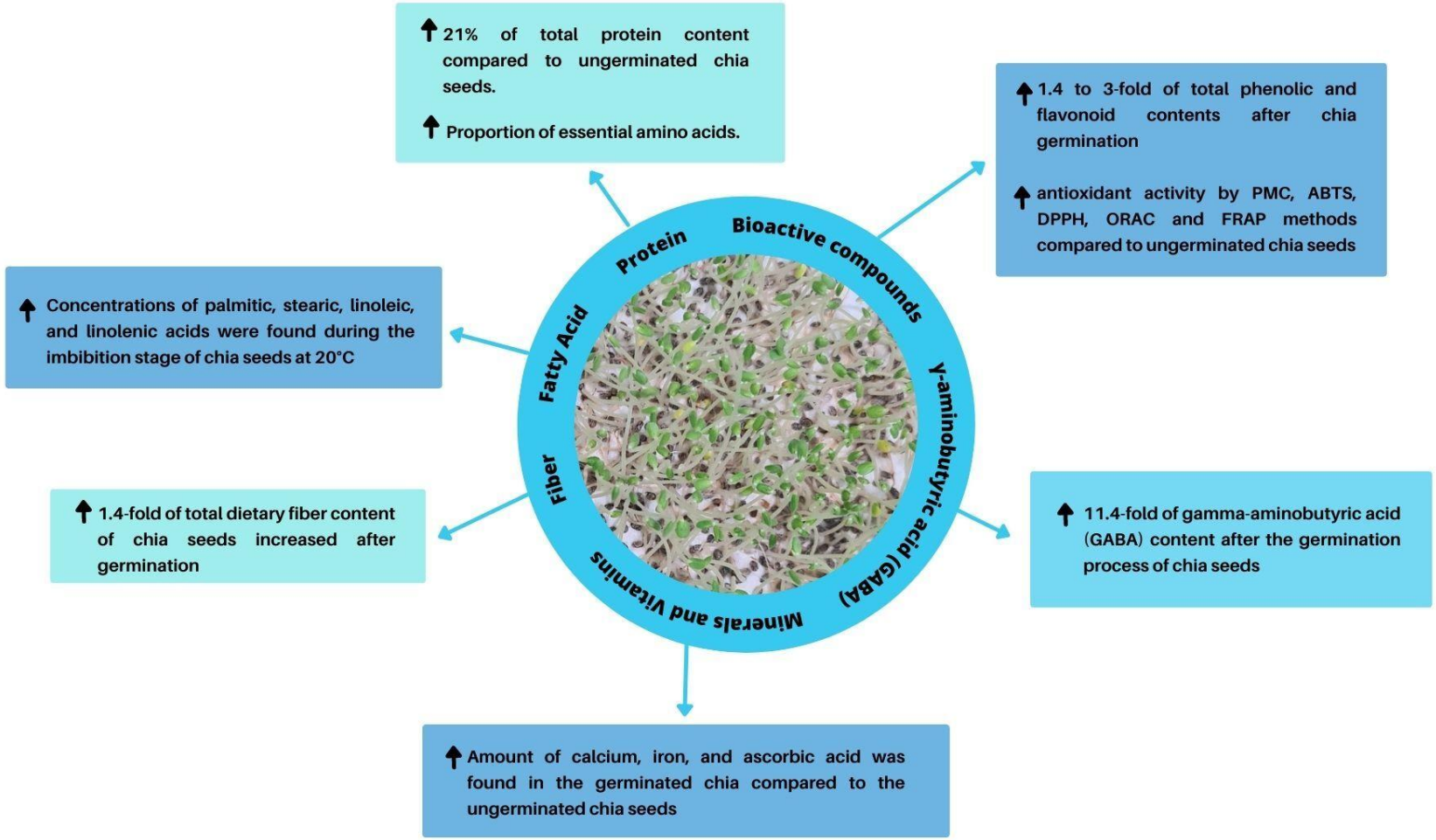

Fig 1. Main changes in the nutritional composition and phenolic compounds in chia sprouts reported in the scientific literature

6 **CAPÍTULO 2**

Centesimal composition, fatty acid profile, phenolic compounds, and antioxidant activity of raw and germinated chia (*Salvia hispanica* L.) seeds

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Abstract

The consumption of chia seeds has become popular due to their beneficial health properties and the germination of chia seeds seems to further enhance these properties. This study aimed to evaluate the changes in the nutritional composition of chia seeds after germination for 3 and 6 days. Proximate composition, fatty acid profile, phenolic content and antioxidant capacity were determined. The indices of lipid quality, atherogenicity, thrombogenicity, and the n-6/n-3 ratio were calculated. Chia sprouts presented a significant increase in minerals, proteins, and a reduction in total lipid content with maintenance of lipid quality. Total phenolic content decreased significantly as germination time increased, but there was a significant increase in the amount of rosmarinic acid. Chia sprouts showed a significant increase in antioxidant potential when compared to raw chia seeds. As a conclusion, the results of this study demonstrated that chia seed germination is a simple, economical, and short-term process capable of improving the nutritional composition of the seeds.

Keywords: Germination, sprout, omega-3 fatty acids, indexes of lipid quality, rosmarinic acid.

Introduction

Chia (*Salvia hispanica* L.) is an herbaceous plant, belonging to the Lamiaceae family, native to the mountainous regions of western and central Mexico and Guatemala. It was cultivated by the Mayas and Aztecs and had great importance because it was used as food, paints, handicrafts and even as medicine [1, 2]. Currently, the commercial cultivation of chia seeds is mainly concentrated in countries such as Mexico, Bolivia, Ecuador, Guatemala, Colombia, Peru, Argentina, and Paraguay [3].

Chia seed is a food that has a high content of antioxidant compounds, protein, dietary fiber and polyunsaturated fatty acids, especially α-linolenic acid, belonging to the omega-3 fatty acid family [4, 5]. In addition, its consumption is associated with increased satiety, regularization of bowel function, decreased blood cholesterol and triacylglycerol, decreased risk of chronic noncommunicable diseases, among others [6-8].

Due to growing concerns about health and the environment, the population is increasingly seeking a healthier lifestyle, opting for fresh foods, and adopting plant-based diets. Therefore, the demand for healthy foods, such as sprouts, grown in natural systems has been increasing [9, 10]. Consumption of sprouts is an ancient practice and their production is simple: it does not require large spaces or many resources for cultivation [11-13]. Sprouts can be defined as the

product obtained from the germination of seeds in water or other media, harvested before the development of true leaves and intended to be eaten whole, including the seeds [14]. Germination is a good way to improve the nutritional value of foods that are frequently consumed, and it can be used in food products [8].

The consumption of sprouts has been increasing as a result of the search for balance in the diet, and although there are still few studies, the literature has reported that the germination of chia seeds results in increased protein content and quality, increased total phenolic compounds and antioxidant capacity [15-17]. Regarding the fatty acid profile, no studies associated with chia sprouts have been found so far. In a study by Cabrera-Santos et al. [18], the changes in fatty acids during hydration of chia seeds at 10°C, 20°C and 30°C was investigated to determine the correlation between fatty acids, temperature, and germination.

Chia seeds are used by the population because of their beneficial properties to health, and the germination process seems to improve these properties. Therefore, scientific research still needs to evaluate the changes that occur in the nutritional value of chia seeds after germination at different times, especially changes related to fatty acid profile. Therefore, this work aimed to investigate the nutritional changes that occur in chia seeds during the germination process.

Results and Discussion

Seed germination is a natural and generally simple process that can improve the nutritional value of food [9, 19]. Regarding chia seeds, its ability to form mucilage when in contact with water, can negatively affect the entry of water and oxygen into the seed and decrease sprout development [20]. In this regard, it is worth noting that the germination process of chia seeds was challenging.

Centesimal composition

The results of the centesimal composition analysis are shown in Table 1. In the present study, there was a significant increase in mineral content after 3 and 6 days of germination compared to ungerminated chia seeds. Increase in mineral content was also found in the study by Gomez-Favela et al. [17], in which germination of chia seeds under optimized conditions (21°C, 156 hours) resulted in more than 59.4% in mineral content. On the other hand, Beltran-Orozco et al. [16] found no increase in ash content after 3 days of germination of chia seeds.

In the present study, there was a significant increase in protein content during chia seed germination, as the 3-day sprouts showed significantly higher values than those found in chia

seeds, and the 6-day sprouts showed higher values than the 3-day sprouts. Gomez-Favela et al. [17], when comparing chia seed flour with sprouted chia flour, found an increase in protein, as in our study, after 156 hours (6.5 days) of germination. The increase in protein content during germination has also been reported in other studies with Australian sweet lupin [21], chickpea, lentil, and yellow pea [22]. The metabolism of non-protein components, such as the reduction of lipids, may have contributed to the proportional increase in protein content [16].

During germination of chia seeds, there was a significant decrease in lipid content compared to raw chia seeds in the present study. This reduction also occurred in other studies that investigated the effect of germination on chia sprouts. For example, Gomez-Favela et al. [17] reported a 55.3% reduction in lipid content in chia flour germinated at 21°C after 6.5 days when compared to chia flour. Beltrán-Orozco et al [16] found a significant increase in lipid content in the first 48 hours of germination (from 37.98 g/100g to 42.16 g/100g); however, lipid content reduced after 72 hours (38.4%) and 96 hours (31%). Other studies conducted on sprouts of Australian sweet lupin [21], wheat (*Triticum aestivum* L.), alfalfa (*Medicago sativa* L.), radish (*Raphanus sativus* L.) and lentil (*Lens culinaris* L.) [23] also reported a reduction in total lipid content during the germination process. This effect can be attributed to increased lipolytic activity, which results in the breakdown of triacylglycerols into fatty acids and glycerol, used as an energy source for seed germination [24, 25].

Fatty acid profile

Table 2 shows the fatty acid composition of the ungerminated chia seeds and the 3- and 6-day-old sprouts. The lipids present in ungerminated chia seeds are mostly composed of polyunsaturated fatty acids, such as α-linolenic acid and linoleic acid [4]. Therefore, it was found that the content of total unsaturated fatty acids, especially polyunsaturated fatty acids, was predominant in all the study samples. For both the ungerminated chia seeds and the 3- and 6-day-old sprouts, α-Linolenic acid (C18:3) was predominant, followed by linoleic acid (C18:2), palmitic acid (C16:0), oleic acid (C18:1), and stearic acid (C18:0). In a study by Imran et al. [26], as in the present study, it was shown that fatty acids present in the highest amount in crude chia oil on day 0 of storage were α-linolenic (60.56 ± 1.22%), linoleic (12.14 ± 0.22%), oleic (8.34 ± 0.19%) and palmitic (6.76 ± 0.15%) acids.

As with flaxseed, chia is an important source of the essential fatty acids α-linolenic (omega-3) and linoleic (omega-6); after vegetable oils, it is the main plant sources of omega-3 fatty acid.

It is noteworthy that essential fatty acids are those polyunsaturated fatty acids that must be supplied by food, since the animal organism is not able to synthesize them. The essential fatty acids α-linolenic and linoleic are precursors of other long-chain polyunsaturated fatty acids, such as the omega-3 fatty acids EPA and DHA and the omega-6 arachidonic fatty acid, all of which have important biological activities, e.g., the synthesis of inflammation mediators with anti-inflammatory (omega-3) and pro-inflammatory (omega-6) action [1, 27, 28].

The results found in this study indicated that there was no significant reduction in the percentage of fatty acids after germination, and that chia sprouts represent an important source of essential fatty acids, especially omega-3, as shown in Table 2. A 50 g (1 ½ cup) serving of chia sprouts (1.97 g on a dry basis) already meets 100 % of the daily value of adequate intake for α-linolenic acid (adequate intake for α-linolenic acid: 1.35 g per day) [29]. A study conducted in Turkey by Ghafoor et al. [30] compared the lipid profile of ungerminated and 4-day germinated chia and found a similar lipid profile to the present study. However, the authors found an increase in saturated fat content and a reduction in omega 3 after germination. This may have occurred due to the difference in seed origin of the studies. In a study conducted by Mattioli et al. [31], flaxseed sprouts (germinated for 3 days) showed a fatty acid profile similar to that of chia sprouts found in the present study.

It is worth noting that studies have shown that omega-3 consumption can reduce the risk of cardiovascular diseases by reducing blood pressure, lowering triacylglycerol levels, improving endothelial function, and reducing inflammation, in addition to having beneficial effects on the prevention and control of neurodegenerative diseases, such as Alzheimer's disease, and on mental health, such as depression and anxiety [32-34].

In this context, chia sprouts can significantly contribute to the improvement of important dietary lipid quality indices: omega-6:omega-3 ratio (n6/n3), index of atherogenicity (IA), and index of thrombogenicity (IT), as discussed below.

Both α-linolenic acid and linoleic acid are metabolized into their corresponding products by common enzymatic systems, i.e., very high amounts of linoleic acid can inhibit the conversion reactions of α-linolenic acid (competitive inhibitory effect), predisposing the body to a proinflammatory state [28, 35]. In this sense, n6/n3 of foods, and consequently of the diet, has great importance for the balance in the metabolism of these fatty acids. Taking into account the value recommended by the Institute of Medicine [29] (n6/n3 = 10) and values found in population studies with different dietary models (n6/n3 = 1.5 for Japanese and n6/n3 = 18 for

North Americans), the literature points out values of n6/n3 ratio in the diet between 1.5 and 3 as adequate [29, 36, 37].

In the present study, the n6/n3 ratio of raw chia seeds and chia sprouts was 0.3. When chia is consumed frequently within a proper diet, this ratio can be considered important for maintaining the proper balance between omega-6 and omega-3 and preventing inflammatory processes, which are related to increased risk for several non-communicable chronic diseases [38-40]. This is due to the fact that omega-6 fatty acid is more abundant in foods than omega-3, favoring an imbalance between the n6/n3 ratio [40].

Regarding IA and IT, lower values are related to better lipid quality of the food. In present study, IA and IT (results not shown in the tables) were 0.09 and 0.06 for the ungerminated chia seeds, 0.10 and 0.06 for the 3-day-old sprouts, and 0.11 and 0.07 for the 6-day-old sprouts, respectively, demonstrating that germination maintained the lipid quality of chia. These values were lower than the ones found by Molska et al. [41], who investigated the changes in fatty acid profile and lipid quality indices in conventional and probiotic added buckwheat sprouts. The authors reported values of 0.17 for IA and 0.32 for IT for both sprouts [41]. As the calculation of these indices is based on the content of omega-6 and omega-3 fatty acids, including the ratio between them, especially in the IT, the lower values found in the present study are supported by the high content of omega-3 and the low n6/n3 ratio.

Phenolic compounds

Table 3 shows the results of total phenolic compound content found in the present study. There was a significant decrease in the levels of total phenolic compounds over the time of chia sprout growth, and the average content of total phenolic compounds in the 6-day chia sprout was significantly lower compared to the 3-day chia sprout. Previous studies have corroborated these findings, such as the study by Miyahira et al. [42], which evaluated the concentration of phenolic compounds in lentil sprouts and found a decrease in phenolic compounds over growth time. Similarly, Liu et al. [43] found a decrease in phenolic compound content after 6 days of the alfalfa germination process. The germination process results in the formation of new structures in plants, and during this process, soluble phenolic compounds tend to bind to carbohydrates and proteins to form new cell walls [44]. In addition, phenolic compounds are easily lost during seed soaking, as they are slightly water soluble [43, 45]. This may account for a reduced amount of these compounds in the soluble fraction [44].

However, other studies have shown different results for the levels of phenolic compounds in chia sprouts. Abdel-Aty et al. [15] reported higher levels of total phenolic compounds in the sprouts compared to the raw seeds. The authors found a significant increase of 4.7-fold in 3 days and 5.8-fold in 6 days, and these values decreased after the seventh day of germination. According to the authors, the increase in the first days of germination may be due to the degradation of phenolic compounds conjugated into soluble phenolic compounds, and with longer germination time, a decrease in free phenolic compounds may occur, as they conjugate with the cell wall again. The authors further argued that the differences in phenolic contents may be mainly due to the type of chia seed cultivar, which probably has more influence than germination conditions, such as temperature, humidity, light, and germination time [15]. Other studies also found increased content of total phenolic compounds in germinated chia sprouts when compared to raw seeds [16, 17].

The extracts of samples of raw and germinated chia seeds for 3 and 6 days showed many substances according to chromatographic analysis by HPLC/DAD and HPLC/MS (Fig 1 supplementary), with two predominant signals - 10.421 min (1) and 11.133 min (2). By co-injection with standard and comparison of ultraviolet and mass spectra, it is suggested that the (2) signal at 11,133 min is from rosmarinic acid. Signal (1) also shows a UV spectrum similar to that of rosmarinic acid, but its mass spectrum shows a molecule with a molecular mass of 522 (detected m/z of 521) suggesting a heteroside of rosmarinic acid. As glycoside is the most abundant heteroside in nature, it is suggested that it is rosmarinic acid 3-O-glycoside.

The final concentrations of rosmarinic acid and its heterosides of the chia extracts in methanol were determined by HPLC-DAD (Table 3). The analyses showed that with increasing time of chia seed germination, there was a significant increase in the concentrations of rosmarinic acid, and a reduction in the concentration of heterosides. The presence of rosmarinic acid in chia seeds and chia sprouts had been previously reported by Motyka et al. [46], who investigated the presence of rosmarinic acid in different parts of chia plants, including seeds and sprouts. The results of the study showed that both chia seeds and sprouts contain rosmarinic acid, with concentrations of 1,27 ± 0.0003 mg/g and 1,34 ± 0.0004 mg/g, respectively. These findings are similar to the results of the present study, which also showed that the sprouts had a significantly higher concentration of rosmarinic acid compared to chia seeds. However, the present study showed higher values of rosmarinic acid in the sprouts.

Another study by Abdel-Aty et al. [15] showed that germination significantly increased the concentration of rosmarinic acid in seeds (0.32 ± 0.01 mg/g) after germinating chia for 7 days

(0.60 ± 0.01 mg/g). This result is interesting since rosmarinic acid has antioxidant, anti-inflammatory, antimicrobial, and antitumor properties [47], and studies have proven that it may have benefits in treating conditions such as cancer, cardiovascular disease, diabetes, Alzheimer's disease, Parkinson's disease, osteoarthritis, asthma, and others [47-49].

Antioxidant capacity

The results of the three methods for analysis of antioxidant activity (ABTS, DPPH and FRAP) (Table 3) show that chia sprouts had higher antioxidant activity compared to chia seeds. In addition, germination time had a positive effect on the antioxidant activity of the sprouts. These results suggest that chia sprouts can neutralize different types of free radicals, as observed in the ABTS and DPPH assays. In addition, chia sprouts also showed the ability to inhibit metal-mediated free radical formation, as evidenced by the FRAP assay.

According to Beltrán-Orozco et al. [16], who investigated the antioxidant activity of chia seeds from Mexico, there was a significant increase in antioxidant activity after four days of germination, as demonstrated by the ABTS, DPPH and FRAP methods. These findings indicate that the germination process exerts a positive effect on the antioxidant activity of chia seeds, which corroborates the results of the present study. The authors attributed the increased antioxidant activity to the increased concentrations of antioxidant compounds such as vitamin C, total phenolics and flavonoids, in addition to the production of new flavonoids and availability of soluble phenolic compounds.

Similar results have been reported by other authors, such as Abdel-Aty et al. [15], who investigated the antioxidant activity of chia and its sprouts using the ABTS and DPPH methods. In this study, the researchers found that chia sprouts exhibited higher antioxidant activity compared to chia seeds. A study conducted with the aim of obtaining functional flour from sprouted chia seeds under optimized conditions investigated the effect of sprouting on antioxidant activity and found that sprouted chia flour was able to neutralize ABTS+ cation radicals about 96.68% more when compared to chia seed flour [17]. The consistency of these findings strengthens the evidence that chia sprouts may exhibit higher antioxidant activity than seeds, and this may play a key role in preventing free radical damage, which is associated with cellular aging and the development of chronic non-communicable diseases [50].

Conclusions

Germination of chia seeds can result in significant changes in the nutritional composition of the sprouts. During the germination process, there was an increase in mineral and protein content. In addition, chia sprouts are an important source of essential fatty acids, especially omega-3, which play essential roles in cardiovascular and brain health. Chia sprouts have a good omega-6:omega-3 ratio, which contributes to a better lipid quality of the diet. Chia sprouts not only demonstrated higher antioxidant activity compared to seeds, but also exhibited the ability to neutralize multiple types of free radicals and inhibit metal-mediated free radical formation, and there was significantly higher antioxidant activity in the 6-day-old sprouts. The results also suggest that the sprouts may be a potential source of rosmarinic acid. However, further studies are needed to evaluate the bioavailability and biological effects of rosmarinic acid present in chia sprouts and its derived products. Therefore, chia sprouts offer nutritional benefits, especially in terms of protein, rosmarinic acid, and essential fatty acids, which makes them a good choice for inclusion in one's diet.

Declarations

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Availability of data and material

Data is available upon request.

Authors' contributions

V.S.C.N.S.: Methodology, Formal analysis, Writing – original draft, Investigation. L.Z.: Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing, Supervision. E.N.F.: Methodology, Formal analysis, Writing – review & editing. M.R.C.M.C.: Methodology, Formal analysis, Writing – review & editing. M.C.: Methodology, Formal

analysis, Writing – review & editing. R.F.M.: Conceptualization, Methodology, Formal analysis, Funding acquisition, Investigation, Writing – review & editing, Supervision.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Statement

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

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Table 1. Basic composition of ungerminated chia seeds, 3-day sprouts and 6-day sprouts per 100 g (dw)

Component (g/100g)	Ungerminated chia seeds	3-day sprouts	6-day sprouts
Protein	19.05 ± 1.11^{c}	21.22 ± 0.74^{b}	24.55 ± 0.15^{a}
Lipids	40.11 ± 0.55^{a}	34.79 ± 0.23^{b}	28.59 ± 0.36^{c}
Carbohydrates	36.47	39.12	41.68
Ash	4.37 ± 0.01^{c}	4.87 ± 0.04^{b}	5.18 ± 0.06^{a}

Values are mean ± standard deviation. Different letters in the same row mean significant differences ($p<0.05$)

Table 2. Percentage of major fatty acids found in ungerminated and germinated chia seeds

Fatty acid (%)	Ungerminated chia seeds	Sprout 3 days	Sprout 6 days
Palmitic acid (C16:0)	7.69±0.15[a]	8.59±0.73[a]	9.27±0.91[a]
Stearic acid (C18:0)	3.68±0.21[a]	4.04±0.24[a]	4.11±0.09[a]
Oleic acid (C18:1)	6.14±0.04[a]	6.39±0.64[a]	6.59±1.02[a]
Linoleic acid LA (C18:2)	18.77±0.35[a]	19.10±0.40[a]	19.22±0.20[a]
α-Linoleic acid ALA (C18:3)	62.66±0.15[a]	60.51±2.17[a]	59.10±2.60[a]
Unsaturated fatty acids	87.91±0.42[a]	86.44±1.06[a]	85.51±1.11[a]
Monounsaturated fatty acids	6.37±0.06[a]	6.70±0.71[a]	7.02±1.23[a]
Polyunsaturated fatty acids	81.55±0.48[a]	79.74±1.77[a]	78.49±2.34[a]
Saturated fatty acids	12.09±0.42[a]	13.56 ± 1.06[a]	14.49 ± 1.11[a]
Omega-3	62.68±0.19[a]	60.57±2.17[a]	59.19±2.60[a]
Omega-6	18.79±0.35[a]	19.13±0.40[a]	19.26± 0.26[a]
Omega-9	6.30±0.08[a]	6.59±0.68[a]	6.77±1.11[a]

Different letters in the same row mean significant differences (p<0.0

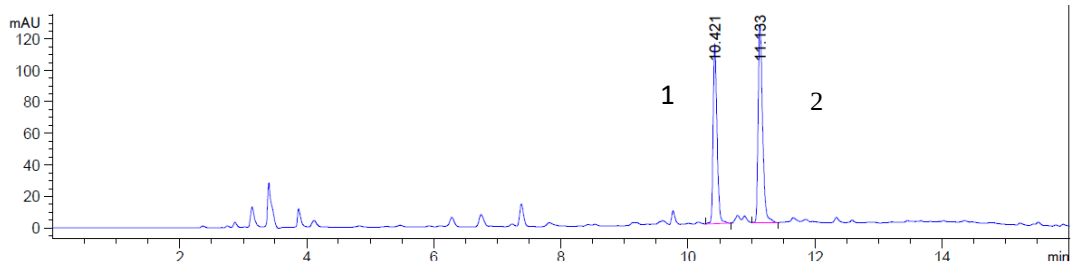
Table 3. Total Phenolic compounds, Rosmarinic acid, Rosmarinic acid 3-O- glycoside and antioxidant activity of the chia seeds, 3-day sprouts and 6-day sprouts, using the ABTS, DPPH and FRAP assays.

Component	Ungerminated chia seeds	3-day sprouts	6-day sprouts
Total phenolics (mg GAE/g)	2.58 ± 0.17[a]	2.24 ± 0.12[b]	1.76 ± 0.07[c]
Rosmarinic acid (mg/g)	0.39 ± 0.004[a]	1.04± 0.004[b]	1.23± 0.004[c]
Rosmarinic acid 3-O- glycoside (mg/g)	0.07 ± 0.004[a]	0.01 ± 0.004[b]	0.008 ± 0.004[c]
Antioxidant activity			
ABTS (.M Trolox/g)	113.98± 0.11[a]	115.29 ± 0.16[b]	115.92 ± 0.19[c]
DPPH (%AA)	59.83 ± 1.92[a]	90.22 ± 0.19[b]	91.47 ± 0.19[b]
FRAP (.M Trolox/g)	2.11 ± 0.22[a]	3.37 ± 0.38[b]	4.74 ± 0.47[c]

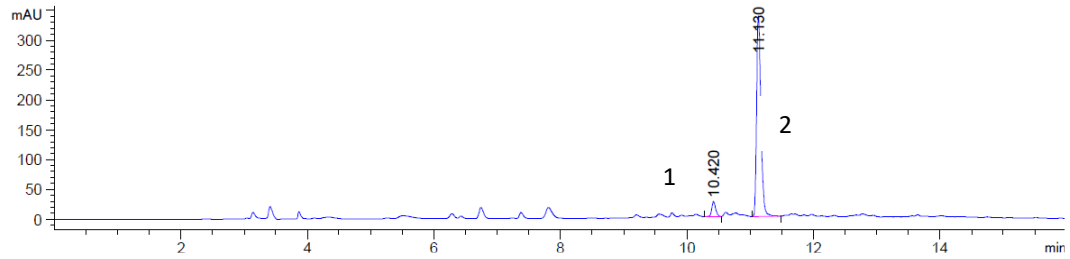
FRAP Ferric-reducing antioxidant potential method, ABTS Trolox equivalent antioxidant capacity, DPPH 1,1-diphenyl-2-picrylhydrazyl free radical, GAE/g gallic acid equivalents per gram. Values are mean ± standard deviation. Different letters in the same row mean significant differences (p<0.05).

Fig 1 supplementary. High-performance liquid chromatogram of chia seed (A), 3-day sprouts seed (B) and 6-day sprouts (C)

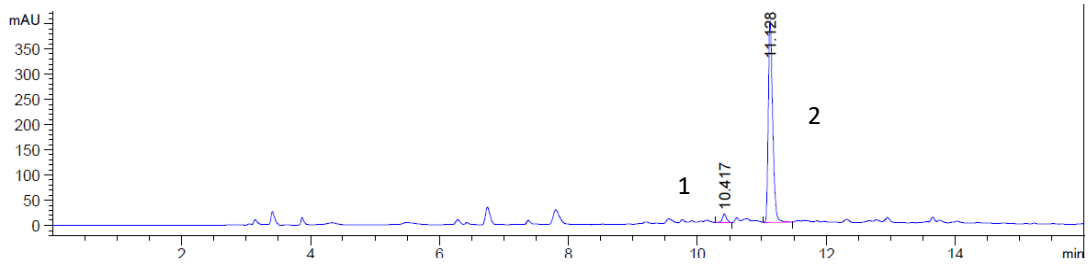
(A)



(B)



(C)



1 - rosmarinic acid 3-O- glycoside; 2 - rosmarinic acid

SUPPLEMENTARY MATERIAL

Materials and Methods

Samples and Germination process

Commercial chia (*Salvia hispanica* L.) seeds were purchased from local markets in Rio de Janeiro, Brazil. The experiments were performed in triplicate with samples of raw chia and chia germinated for 3 and 6 days. The germination process was performed according to

Abdel-Aty et al. [1]. Chia seeds were sanitized in sodium hypochlorite solution for 10 min and then washed several times with filtered water. The seeds were then placed into a plastic tray containing seed germination paper. Chia seed germination was performed in the dark and at room temperature (25°- 30° C). Filtered water was sprayed daily in the morning and evening. After germination of 10 g of chia seeds, the 3-day and 6-day yields were 47.5 g and 52.47 g, respectively. The 3-day and 6-day germination samples were frozen at - 80°C [1], freeze-dried (Liofilizer model L-101, Liotop brand), ground to a fine powder and stored at -80°C for further analysis.

Proximate analysis

Centesimal composition analyses were performed according to the official methods described by AOAC [2]. Briefly, moisture was determined by loss through desiccation using direct drying in an oven at 105°C; fixed mineral residue, by incineration in a muffle furnace at 550 – 570°C, and protein, by the Kjeldahl method using 6.25 as the conversion factor of total nitrogen into protein. Total lipids were determined by Soxhlet. Carbohydrates were determined by difference. The results were expressed on a dry mass basis.

Fatty Acid Analysis

Total lipids were extracted using acid hydrolysis methods. Approximately 100mg of pyrogallic acid was added during the analysis to minimize the oxidative degradation of fatty acids. The undecanoic triglyceride (C11:0) was added as an internal standard. Fat was extracted using ether and methylated to fatty acid methyl esters (FAME) by reaction with boron trifluoride (BF3) in methanol.

The lipid fraction was subjected to a transesterification reaction and the recovered fatty acid methyl esters were analyzed by gas chromatography (Thermo Fisher) coupled with a Flame Ionization Detector (FID). A TR-FAME capillary column (120 m length × 0.25 mm i.d., 0,25 µm film thickness, part number: 260M166L Thermo Fisher) which was used for the GC system. The oven temperature was set as follows: from 100 ◦C to 240 ◦C with 3◦C/min. The injector temperature was 225ºC ◦C in split mode. Helium was used as carrier gas at a linear flow velocity [2]. FAMEs were quantitatively measured by using C11:0 internal standard. Total fat was calculated as the sum of all fatty acids. Saturated and monounsaturated fats were calculated as the sum of their respective fatty acids. Fatty acid analyses were performed by duplicate and the results were expressed as a percentage of fatty acids relative to total fat.

Indices of lipid quality

The index of atherogenicity (IA), index of thrombogenicity (IT) and n6/n3 ratio were calculated based on fatty acid profile data. IA and IT were calculated using the equations described by Ulbricht & Southgate [3].

Extraction procedures

The extraction methodology for the phenolic compounds followed the protocol described by Abdel-Aty et al. [1], with modifications. One gram of chia (germinated or raw freeze-dried) was suspended in 10 mL of a methanol/water solution (80:20, v/v). The samples were placed under stirring for 24 h at room temperature (20°-25°C). Afterwards, the samples were placed in a centrifuge (4000 rpm) cooled at 4o C for 30 min. At the end, the supernatant was removed and placed in a Falcon tube at -20oC for further analysis. Phenolic compounds and antioxidant activity were determined in the extracts, and the results were expressed on a dry mass basis.

Total phenolic content (TPC)

The Folin-Ciocalteu method was applied according to Singleton et al. [4] to determine the TPC of germinated and raw chia. In a 96-well microplate, 20µL of the sample or standard and 100µL of 10% Folin-Ciocalteau were added. After 5 minutes, 75µL of 7.5% sodium carbonate was added, and the plate contents were gently shaken for homogenization. The plate was stored for reaction for 40 minutes in the dark, with subsequent reading at 740nm (Biochrom Asys UVM 340). The results were expressed as milligrams of gallic acid equivalents per gram (mg GAE/g) as dry weight (DW).

Identification of phenolic compounds by High Performance Liquid Chromatography (HPLC)

All reagents (analytical grade) and solvents (HPLC grade) were purchased from Sigma (Brazil). All of the standards of phenolic compounds being used were also purchased from Sigma (> 98% purity): apigenin, aromadendrin, caffeic acid, catechin, carnosol, chlorogenic acid, chrysin, trans-cinnamic acid, coniferyl aldehyde, o-coumaric acid, eriodyctiol, ferulic acid, fustin, galangin, gallic acid, hispidulin isoorientin, isoquercitrin, kaempferol, luteolin, 4-methylumbelliferone, naringenin, naringin, pinocembrin, protocatechuic acid, quercetin, rosmarinic acid, resveratrol rutin, scopoletin, sinapaldehyde, sinapic acid, sodium salicylate, syringaldehyde, syringic acid, taxifolin, umbelliferone, vanillic acid, vanillin, vitexin.

The characterization of phenolic compounds in the raw and germinated chia seeds for 3 and 6 days was performed by HPLC (Agilent - 1200 infinity) with an ultraviolet detector by diode arrangement (1260 DAD G4212B) as described by Abdel-Aty et al. [1], with modifications. The separation was performed using a Hypersil Gold – C18 column (250 x 4.6 mm, 5 µm

particle) and the compounds were identified by comparing the peak retention times with those obtained by the co-injection of pure standards and/or by LC-MS analysis (Perkin Elmer Altus A-30) using an Altus UPLC BEH C18 column (150 mm x 2.1 mm, with a particle size of 1.7 µm). Sample injection volume was 10 µL.

Chromatographic conditions: acetonitrile (A) and ultra-pure water (MilliQ) acidified with 0.1% formic acid and pH 3.2 (B). At the beginning, the ratio was 5:95 (A:B), with a gradual change: in 4 minutes, the ratio was 15:85 (A:B), in 9 minutes 50:50, and finally, in 13 minutes, the ratio inverted to 100:0 (A:B), remaining isocratic up to 15 minutes and returning to the initial condition in 16 minutes. Between runs, 5 minutes were allowed for column reconditioning. The flow rate was 0.3 mL.min-1 and the column temperature was 40°C during the analysis.

Calibration curve and linearity by rosmarinic acid

By co-injection in the HPLC-DAD and by the mass spectrum obtained by LCMS, it could be confirmed that rosmarinic acid is the most abundant phenolic compound in chia extracts. Then, the calibration curve was determined with this substance. Rosmarinic acid was dissolved in methanol and diluted to 20-200 µg.mL-1 concentration ranges for establishing standard curve. The standard curve was prepared by plotting the average of peak areas versus the concentrations of each compound. The validation of rosmarinic acid was performed, with tests of selectivity, linearity, limits of detection and quantification, accuracy and intermediate precision determined and statistically treated by the Action Stat software. All results were considered within the limits established by the Brazilian legislation [5].

Antioxidant activity

Three different methods were used to evaluate the antioxidant capacity of germinated and raw chia. All reactions were performed in a 96-well microplate and read by spectrophotometry (Biochrom Asys UVM 340), using the calibration curves and the corresponding straight-line equation. Antioxidant activity was determined by the ferric-reducing antioxidant potential method (FRAP) as described by Müller et al. [6], with reading at 595nm, and the results were expressed in equivalent to ferrous sulfate (µM). The trolox equivalent antioxidant capacity (ABTS) and scavenging effect on 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) assays were determined according to Al-Duais et al. [7] at a reading of 730nm for ABTS and 517nm for DPPH.

Statistical analysis

The analyses were performed in triplicate for all determinations. The results were presented as mean and standard deviation. The data were statistically treated by analysis of variance (ANOVA) using Tukey's test to verify significant differences. The significant level adopted was 5% ($p < 0.05$), using GraphPad Prism 9 statistical software.

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7 **CAPÍTULO 3**

Effect of sanitization with sodium hypochlorite on microbiological quality in home production of lentil, wheat, and chia sprouts

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ABSTRACT

Owing to the increase in the number of chronic non-communicable diseases, the inclusion of healthier foods in the diet is becoming increasingly frequent. In this sense, the consumption of sprouts can be considered as a good alternative because they are easy to germinate, are tasty, and have high nutritional value. However, their consumption has been related to several food outbreaks, since they are foods susceptible to microbial contamination. This work aimed to evaluate the microbiological quality of lentil sprouts and wheat sprouts germinated in a domestic environment, and the effectiveness of the sprout and seed sanitization process. The sanitization process with sodium hypochlorite was performed on seeds before germination and on sprouts in wheat, lentil, and chia samples. Total coliforms, *E. coli*, mesophilic aerobes and *Bacillus cereus* were determined, and the presence of *Salmonella* spp. and *Listeria monocytogenes* were investigated. No strains of *Salmonella* spp., *E. coli* and *Listeria monocytogenes* were found. However, a high microbial load of total coliforms was found in the sprouts. *Bacillus cereus* was found only in the wheat sprouts and chia samples. The sanitization process with sodium hypochlorite was able to reduce the microbial load of the sprouts, but the load remained high. Therefore, ideally, seeds should be marketed only when they are suitable for home germination after going through a more efficient decontamination process. In addition, competent authorities are supposed to create and widely disseminate a document of good production practices for safer domestic cultivation of sprouts.

Keywords: Bacteria; Germination; Seed disinfection; Total coliforms; chemical decontamination

1. Introduction

Some countries, such as Brazil, the United States, and Australia have nutritional guidelines which advocate that the consumption of fresh or minimally processed foods should be the basis for a nutritionally balanced, tasty, and sustainable diet, and this group includes food such as grains, tubers, roots, vegetables, and fruits (Brasil, 2014; NHMRC, 2013; USDA, 2020). Owing to an increase in the number of individuals with chronic non-communicable diseases over the years, consumption of these foods is increasingly important, since a plant-based diet is a preventive factor for these diseases (Barros et al., 2021).

However, people's current lifestyle, characterized by lack of time, is one of the main reasons for an inadequate diet (Menezes and Maldonado, 2015). Although cooking requires time and planning, it can contribute to better diet quality. Preparing and cooking one's own food helps the transition to and maintenance of a healthier lifestyle (Oliveira and Castro, 2022; Brasil, 2014).

Grains are staple foods that are widespread and consumed worldwide; they have an optimal nutritional composition and bring beneficial health effects (Aloo et al., 2021). Consumption of sprouts has become popular worldwide because consumers have been searching for foods with high nutrient content. The sprouting process leads to several changes in the nutritional composition of grains, e.g., it improves digestibility, increases nutritional value, reduces antinutritional factors, and favors the accessibility of nutrients (Miyahira et al., 2021). Peñas and Martínez-Villaluenga (2020) stated that germination reactivates seed metabolism, inducing the degradation of macronutrients and antinutritional compounds, and is also able to form bioactive compounds that are beneficial to health. Salgado et al. (2021) reported that an increase in protein content, dietary fiber, phenolic compounds, and decrease in antinutritional factors occurred after germination in chia sprouts, for example (Salgado et al, 2021). In wheat sprouts, germination can increase antioxidant activity (Miyahira et al., 2022). In lentils, this process can increase the concentration of protein and nutrients such as iron, zinc, and manganese (Santos et al., 2020). Thus, the cultivation of sprouts in the home environment that does not require a large space, and it consists of simple, economical, and sustainable steps. Moreover, it is an excellent strategy to encourage consumers to participate in the entire process of production of this highly nutritious food until the moment it actually available for consumption (Ebert, 2022).

Despite the demonstrated benefits of grain germination, the conditions of this process are favorable to the proliferation of pathogenic microorganisms present in the grain. Increased moisture, ambient temperature, and high nutrient availability result in an ideal scenario for microbial growth (Brankatschk et al., 2014; Ding et al., 2013; Iacumin and Comi, 2019). Foodborne outbreaks have been associated with sprout consumption in different parts of the world between 1988 and 2020, totaling about 15,342 cases, 313 hospitalizations, and 61 deaths (Carstens et al., 2019; Miyahira and Antunes, 2021).

In order to minimize the risks involved in sprout production, the U.S. Food and Drug Administration (FDA), along with Institute for Food Safety and Health (IFSH), created the Sprout Safety Alliance (SSA), a public-private alliance that develops training programs and implementation of best practices for safe sprout production (Sprout Safety Alliance/FDA). Proper decontamination treatments should inactivate pathogenic microorganisms while preserving seed viability, germination, and vigor (NACMCF, 1999). Although complete elimination of bacterial contamination by seed disinfection treatments in sprout production is very difficult to achieve (EFSA, 2011), studies have evaluated different methods (chemical, biological and physical methods) of seed disinfection and the impact of these processes on germination capacity (Ding et al., 2013; Sikin et al., 2013). According to Gilbert et al. (2023), heat treatments that significantly reduce microbial growth also decrease seed germination capacity and are therefore not eligible for seed disinfection. Among the various chemical sanitizers recommended, sodium hypochlorite and hypochlorous acid were the most effective in reducing bacterial load by at least 5 logarithmic units without adversely affecting seed germination (Gilbert et al., 2023).

Considering that germination increases the nutritional value of grains, and germination conditions may be conducive to the development of pathogenic microorganisms, the microbiological contamination of sprouts and the efficiency of chemical sanitization of seeds and sprouts need to be evaluated for further development of actions to support regulations that ensure the safe production of sprouts. The reason is that sprouts are being increasingly grown in domestic environments and used in salads or food preparations that do not undergo any heat treatment for pathogen reduction. Thus, this study aimed to evaluate the microbiological quality of lentil, wheat and chia sprouts produced in the home environment, as well as the effectiveness of the sanitization process with sodium hypochlorite on the sprouts and seeds.

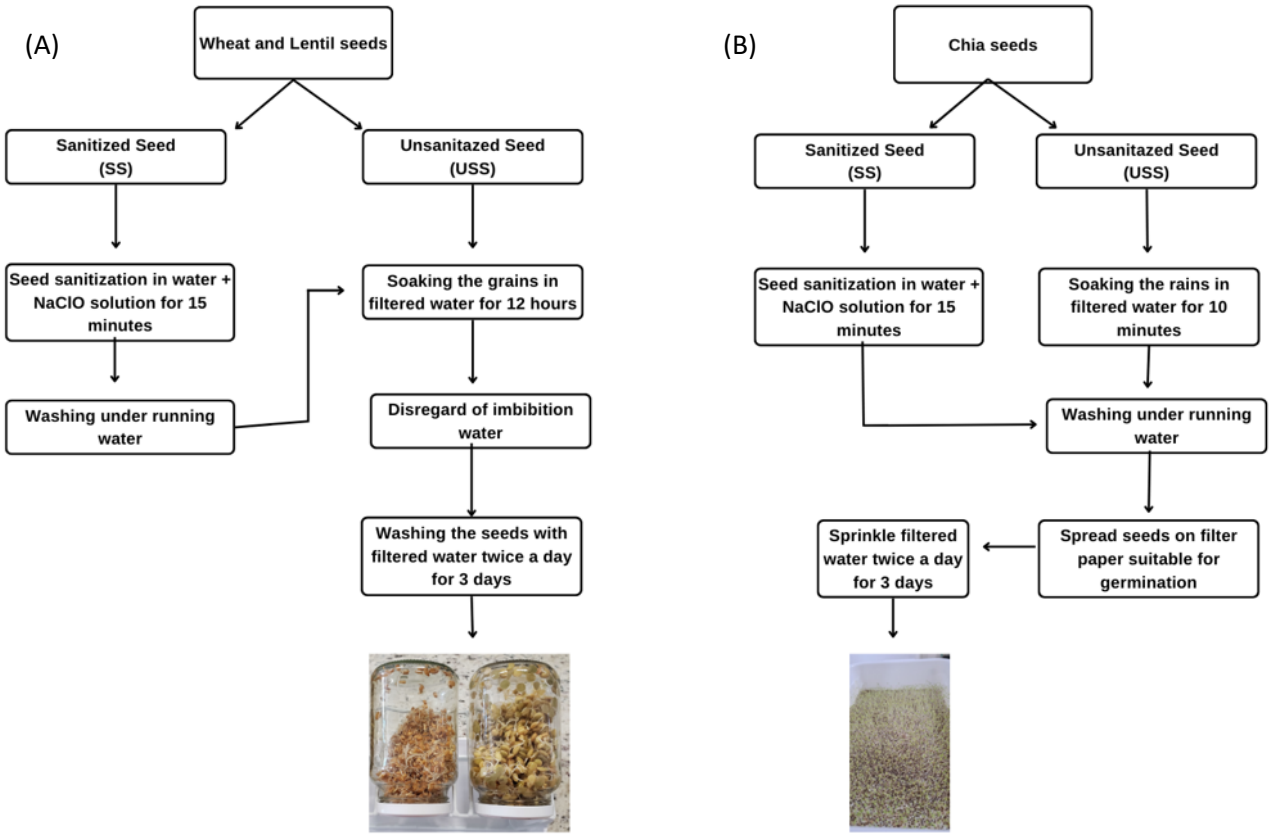
2. **Materials and Methods**

2.1. *Samples and germination/sanitization process*

Seeds of lentil (*Lens culinaris* L.), wheat (*Triticum aestivum* L.) and chia (*Salvia hispanica* L.) were purchased from local markets in Rio de Janeiro, Brazil. For germination, the seeds were divided into sanitized seed (SS) and unsanitized seed (USS) groups. After germination, the SS and USS groups were subdivided into four groups: unsanitized seed and unsanitized sprout (USS/USP), unsanitized seed and sanitized sprout (USS/SSP), sanitized seed and unsanitized sprout (SS/USP), and sanitized seed and sanitized sprout (SS/SSP). Figure 1 shows a sanitization/germination flowchart. The germination process was repeated five times for each of the four seed/sprout treatment groups, totaling 60 samples, 20 of lentil, 20 of wheat, and 20 of chia sprouts. Germination of lentil and wheat grains was performed according to Miyahira et al (2022). To this end, the grains were washed in running water, then soaked in filtered water for 12 hours at room temperature (25 °C). Then, the water was drained and the grains were placed in tilted glass jars with perforated lids. The germination process of chia seeds was performed according to Abdel-Aty et al. (2021). The chia seeds were washed with filtered water. Then, the seeds were placed inside a plastic tray containing seed germination paper. Chia seed germination was performed in the dark and at room temperature (25°- 30° C). Filtered water was sprayed daily in the morning and evening for 72 hours.

The sanitization process performed before (on the seeds) and after germination (on the sprouts) was the same. A solution of water (1 liter) added to 10 mL of sodium hypochlorite at a concentration of 2% was prepared in a sterile container for 15 minutes, as specified on the product label by the manufacturer. Microbiological analyses were performed on the sprouts from March to October 2022, immediately after seed germination.

Fig 1: Flowchart of sanitization/germination of lentil, wheat (A) and Chia (B) seeds



2.2. Microbiological analysis

2.2.1. Serial dilutions used for mesophilic aerobic bacteria, Bacillus cereus, total coliforms, and Escherichia coli analyses

10 g of the sample was homogenized with 90 mL of 0.1% peptone water (10^{-1} dilution). Next, serial dilutions were made with 1 mL of the 10^{-1} dilution into a tube with 9 mL of 0.1% peptone water, performing the 10^{-2} dilution up to the 10-^{7} dilution (APHA, 2015).

2.2.1.1. Determination of Total Coliforms and Escherichia coli

1 mL of the dilutions was inoculated onto 3MTM PetrifilmTM plates. The plates were incubated at 35 ± 1 ºC for 24 to 48 ± 2h with the transparent side facing up. After this period, the typical colonies of total coliforms (red colonies with gas) and *E. coli* (blue or blue-red colonies with gas) were counted.

2.2.1.2. Determination of Total Aerobic Mesophilic Bacteria

For mesophilic aerobic bacteria analysis, the pour plate technique was used, with plating of the dilutions on Standard Counting Agar (SCA) culture medium and incubation at 35°C± 2 for 48h (APHA, 2015).

2.2.1.3. Determination of Bacillus cereus

For determination of Bacillus cereus, the spread plate technique was used as described by ISO method 7932 (7932. 2004) with modifications. 0.1 mL of the 10^{-1}, 10^{-2}, and 10^{-3} dilutions was transferred to plates containing MYP medium and incubated at 30 ± 1°C for 18 to 24 h to check for typical colonies. The typical colonies were confirmed by the hemolysis test on blood agar n.2 supplemented with sheep blood and incubated at 30°C for 24h.

2.2.2. Detection of Listeria monocytogenes

For detection of *Listeria monocytogenes* according to FDA's Bacteriological Analytical Manual (Hitchins et al., 2022), 25g of the sample was homogenized in 225 mL of Listeria Enrichment Broth (BLEB) and incubated at 30°C/4h. Then the selective agents (0.455ml 0.5% acrylflavine, 1.8mL 0.5% nalidixic acid solution, 1.15mL 1% cycloheximide solution) were added and incubated at 30°C/44h. After incubation, one well from the selective enrichment vial was streaked onto an Oxford Agar (OXA) plate and the plates were incubated at 35°C/24-48h to check for typical colonies. When present, typical colonies were streaked on Soybean Trypticase Agar plates supplemented with 0.6% yeast extract (TSA-YE) and incubated at 30°C/24-48h. After the incubation period, for confirmation purposes, the catalase, motility, and hemolysis tests were performed on blood agar n. 2 supplemented with sheep blood (35ºC/24h). Positive colonies in the tests were stored in skim milk at - 20 °C for later identification by MALDI-TOF.

2.2.3. Detection of Salmonella spp.

For detection of *Salmonella* spp., 25g of the sample was homogenized in 225 mL of 1% Peptone Water and incubated at 37°C/24h. Then, 1 mL of the pre-enriched sample was transferred to tubes with the selective media Tetrationate Broth (TT) and Selenite Cystine Broth

(SC) and incubated again under the same conditions as before. After incubation in the selective media, one batch of each broth was streaked onto Hecktoen's Enteric Agar (HE) and Xylose Lysine Agar (XLD) plates to check for typical colonies. The plates were incubated at 37°C/24h. Typical colonies, when present, were inoculated into tubes slanted with Lysine Iron Agar (LIA) and Triple Iron Sugar Agar (TSI) for confirmation (FDA, 2007). Colonies with characteristic growth on LIA and TSI media were stored in skim milk at - 20 ºC for further identification by MALDI-TOF.

2.3. Maldi-Tof

The typical isolated *Salmonella* spp and *Listeria monocytogenes* colonies were stored and frozen in skim milk at -20 ºC and then identified by mass spectrometry (MALDI-TOF), according to the standard extraction protocol, using formic acid as recommended (Bruker, 2015), at the Medical Microbiology Research Laboratory of the Paulo de Góes Microbiology Institute at the Federal University of Rio de Janeiro.

2.4. Analysis of the water used in germination

To collect water samples, sterilized glass bottles with 250 mL capacity were used, with the addition of 0.02 g (or 1.0 mL of 2% solution) of sodium thiosulfate for each 100 mL. The potable water used in the germination process and the water used to rinse the samples after the sanitization process were collected. The method was based on the American Public Health Association (APHA, 2015). The water samples were tested for total and thermotolerant coliforms using the Most Probable Number (MPN) technique.

2.5. Data Analysis

The data collected from the microbiological analysis were analyzed and expressed in log CFU/g for total coliforms, *Escherichia coli*, *Bacillus cereus* and total mesophilic aerobic bacteria, and in presence/absence of *Salmonella* spp. and *Listeria monocytogenes* in 25g of the sample.

The results were presented as means and standard deviations calculated by Microsoft Excel. Analysis of Variance (ANOVA) and Tukey's test were applied to determine statistically

significant differences between the types of sanitization treatment, with a significance level of 5% ($p < 0.05$), using statistical software GraphPad Prism 9.

3. **Results and Discussion**

The germination process is favorable to microbial growth, and some outbreaks involving sprouts have been reported in different parts of the world. The most recent were reported in the year 2020, with 51 cases, with 3 hospitalizations, related to *E. coli* involving clover sprouts (CDC, 2020) and in the year 2022, 63 people from eight states in the United States were infected with *Salmonella typhimurium* strain after consuming raw alfalfa sprouts. It is estimated that the number of people infected in this latest outbreak was probably higher than reported because people recovered without medical care and without being tested for *Salmonella* (CDC, 2023a).

Despite reports involving outbreaks with sprouts, a study, conducted in Brazil and published in 2023, showed that biohazard is not one of the main topics that consumers relate to this food. The study evaluated the perception of 315 Brazilian consumers about sprouts and showed that in a word association questionnaire, the 4 categories most mentioned by the participants were “sprouts”, “healthiness”, “environment” and “affective memory”, while the category “biological risk” was the least mentioned. This result shows that few people related the consumption of sprouts with a negative aspect, such as foodborne diseases (FBD) (Miyahira et al., 2023). It is particularly relevant to assess the microbiological quality in the sprout production process to ensure consumption of a safe food, because dried seeds contain between 10^3 and 10^5 nonpathogenic bacteria per g, which multiply rapidly during germination, and sprouts can contain between 10^8 and 10^9 nonpathogenic bacteria per g until consumption (NACMCF, 1999). This very high bacterial population can make it difficult to detect pathogenic bacteria in sprouts. The location of bacteria on the surface of seeds/sprouts, their internalization potential, and their ability to adhere to seed/sprout tissues, are also important factors that will influence the effectiveness of intervention strategies such as washing and decontamination (ESFA, 2011).

Table 1. Results of microbiological analyses of lentil, wheat and chia sprouts before and after the seed and sprout sanitization process.

Sample	Microorganisms	USS/USP	USS/SSP	SS/USP	SS/SSP
		Mean ± SD			
Lentil	Total Coliform (log CFU /g)	8.55±0.09^{a}	7.99±0.32^{b}	8.63±0.02^{a}	7.32±0.13^{c}
	Escherichia coli (log CFU /g)	<1	<1	<1	<1
	Aerobic mesophilic (log CFU / g)	8.88±0.09^{b}	8.17±0.24^{c}	9.38±0.09^{a}	8.41±0.12^{c}
	Bacillus cereus (log CFU /g)	<2	<2	<2	<2
	Listeria monocytogenes/25g	Absence	Absence	Absence	Absence
	Salmonella spp./25g	Absence	Absence	Absence	Absence
Wheat	Total Coliform (log CFU /g)	8.41±0.30^{ab}	7.84±0.05^{c}	8.60±0.31^{a}	8.07±0.12^{b} ^{c}
	Escherichia coli (log CFU /g)	<1	<1	<1	<1
	Aerobic mesophilic (log CFU /g)	9.12±0.10^{a}	8.74±0.25^{a}	8.64±0.41^{a}	7.93±0.48^{b}
	Bacillus cereus (log CFU /g)	5.46±0.51^{ab}	4.88±0.51^{ab}	5.61±0.62^{a}	4.65±0.47^{b}
	Listeria monocytogenes/25g	Absence	Absence	Absence	Absence
	Salmonella spp./25g	Absence	Absence	Absence	Absence
Chia	Total Coliform (log CFU /g)	8.54±0.61^{a}	7.59±0.30^{ab}	8.46 ±0.14^{a}	6.57±1.65^{b}
	Escherichia coli (log CFU /g)	<1	<1	<1	<1
	Aerobic mesophilic (log CFU /g)	7.52±0.25^{a}	7.86±0.70^{b}	8.72±0.15^{a}	7.09±0.27^{c}
	Bacillus cereus (log CFU /g)	6.03±1.31^{a}	5.90±1.02^{ab}	5.71±0.66^{ab}	4.22±0.29^{b}
	Listeria monocytogenes/25g	Absence	Absence	Absence	Absence
	Salmonella spp./25g	Absence	Absence	Absence	Absence

Caption: USS: unsanitized seed, SS: sanitized seed, USP: unsanitized sprout, SSP: sanitized sprout

Identical letters in the same row indicate that there was no significant difference ($p<0.05$) in the samples

SD: Standard Deviation; CFU: Colony Forming Units; g: gram

3.1. Total Coliforms

Outbreaks of foodborne diseases related to the consumption of fresh products are usually associated with the presence of pathogenic microorganisms from fecal contamination. Therefore, the determination of total coliforms can be considered as a good indicator of hygiene, although not always indicating the presence of pathogens (Seo et al., 2010).

In the present study, the results for total coliforms, in the lentil samples in which the sprouts were sanitized (USS/SSP), show that the values found were significantly lower than those in which the sprouts were not sanitized (USS/USP) (Table 1). In wheat, it was found that in the samples in which the sprouts had not been sanitized (USS/USP and SS/USP), there was a significantly higher microbial load of total coliforms than those that had been sanitized (USS/SSP and SS/SSP) (Table 1). For chia, it was found that the microbial load of total coliforms was significantly lower in the samples in which seeds and sprout had been sanitized when compared to the samples in which the sprout had not been sanitized, regardless of seed sanitization. It can be inferred that sanitization of the sprouts significantly reduced the number of total coliforms in the three types of study grains; however, it was found that the microbial load remained high, with values higher than 6.57±1.65 log CFU/g. It is worth noting that when only the seed was sanitized, there was no significant reduction in the number of total coliforms in the three study sprouts.

Martínez-Villaluenga et al. (2008) conducted a study to evaluate the microbiological quality of broccoli and radish sprouts germinated for up to five days. Prior to germination, seeds were sanitized with 7% sodium hypochlorite for 30 minutes, washed and soaked with distilled water and then placed in a seed germinator. Total coliforms were investigated, and an average value of 9.50 log CFU/g was found in broccoli seeds after germination. In the radish seeds, an average value of 8.04 log CFU/g was found. The values were similar to the one found in the present study, which is indicative of a high microbial load in the sprouts.

Another study by Tornuk et al. (2011) determined the number of total coliforms in wheat seeds and sprouts germinated for 9 days under different conditions of relative humidity (RH) (90% and 95%) and temperatures (18 °C, 20 °C, and 22 °C), and investigated the disinfection capacity of sodium hypochlorite (NaOCl) (100, 200, and 400 ppm) and hydrogen peroxide (H_2O_2) (3% and 6%). The authors found a significant increase in total coliforms after germination and reported that increasing concentrations of NaOCl and H_2O_2 resulted in reductions in the total coliform population; there were greater reductions when seeds had been

soaked in 400 ppm NaOCl for 30 minutes and then germinated at 18 °C and 90% RH. It is worth noting that the authors also found that sodium hypochlorite at 200ppm was also able to reduce total coliforms significantly by 0.54 log CFU/g. This result was different from that of the present study, which found no significant difference in the number of total coliforms after sanitizing only the seeds.

3.2. Escherichia coli

There are several species of pathogenic *Escherichia coli* (*E. coli*) that can cause diseases, for example, Shiga toxin-producing *E. coli* (STEC), which is involved in some cases of sprout-related food outbreaks (EFSA, 2011). In Germany, in 2011, there was an outbreak linked to sprouted seeds that was associated with an *E. coli* species which acquired the ability to produce the Shiga toxin (Santos et al., 2020). This same strain was linked to a smaller outbreak in France in the same year (Gault et al., 2011; Rasko et al., 2011).

The European Commission (2005) - the regulation that provides for microbiological standards - establishes as hygiene criteria for ready-to-eat fruits and vegetables, that in 5 samples of a batch, only two of these may present *E. coli* values between 10^2 and 10^3 CFU/g. In the Brazilian legislation (Brasil, 2022), the ready-to-eat foods establishes as a criterion that in 5 samples from a batch, only two of these may present *E. coli* values between 10 and 20 CFU/g.

In the present study, the presence of characteristic *E. coli* colonies was not found in any of the study samples. For this reason, it was not possible to evaluate the effectiveness of the sanitization process in reducing the amount of *E. coli* in the samples of this study. However, Tornuk et al. (2011), when analyzing the microbial load of wheat seeds and its changes during germination, found that there was an increase in the number of *E. coli* specimens during germination in different conditions of humidity and temperature, reaching up to 6.37 log CFU/g at 90% humidity and 22°C temperature, and 7.17 log CFU/g at 95% humidity and 22°C temperature. The authors also found that sanitization with sodium hypochlorite or hydrogen peroxide at different concentrations was able to significantly reduce the presence of this microorganism.

Jeddi et al. (2014) performed microbial evaluation of vegetables and sprouts of wheat and mung bean bagged in a supermarket chain, and found the presence of *E. coli* in 12 of 64

sprout samples, corresponding to 18.7% of the study samples. Kim and Cheig (2021) evaluated the microbiological contamination of minimally processed products in Korea, and they found an average of 1 log CFU/g of *E. coli* in 7 samples of mixed sprouts, which was considered to be within the acceptable limit according to the Korea Food Code standards. A literature review by Sikin et al. (2013) showed that several treatments are effective in decreasing the microbiological load of *E. coli* in seeds and sprouts; however, some sanitizers, such as organic acids, can impair the germination process or its sensory characteristic.

3.3. Aerobic mesophilic bacteria

Fresh produce has varied and complex microflora. Interactions with microflora may be able to interfere with the growth of pathogens (Matthews et al., 2014).

The results of Aerobic mesophilic (AM) in the lentil samples showed that the sanitized sprouts had statistically lower values than the unsanitized ones (Table 1), regardless of seed sanitization. Therefore, there was no reduction in the amount of AM when the seed was sanitized before sprouting, and the potential for AM internalization in lentil seeds and their structure may have influenced this result (EFSA, 2011). By contrast, in wheat, only SS/SSP had a significant reduction of AM when compared to the other samples (Table 1), i.e., the use of sanitization, only when performed simultaneously on the seeds and the sprouts, was effective in reducing the microbial load in wheat sprouts. In chia, sprout sanitization significantly reduced the amount of AM, while sanitization of the seeds alone was not able to reduce AM significantly (Table 1).

Saroj et al. (2006) reported that vegetable seeds can contain less than 2 log CFU/g of mesophilic aerobic bacteria and that this population of microorganisms can increase rapidly during germination owing to favorable conditions for bacterial growth. The mean AM count found in the present study was similar to the one found by Seow et al. (2012), who reported mean AM counts in bean sprouts sold in Singapore of 8.0 log CFU/g. The same occurred with the study conducted by Tango et al. (2018): the authors found the value of 9.35 log CFU/g of AM in mixed sprouts sold in Korea that had not been disinfected for microorganism reduction. Tornuk et al. (2011) compared the effectiveness of chemical sanitization in reducing AM and found that treating wheat seeds with 400 ppm NaOCl reduced AM count by 0.29 log compared

to control, and further found a 1.46 log reduction in sprouts after seed sanitization when compared to sprouts without seed sanitization. The reduction in the amount of AM in the study of Tornuk et al. (2011) was much greater than that found in wheat in the present study, which was 0.48 log between USS/USP and SS/USP samples. The authors explained that chlorine-based sanitizers affect microbial cells in various ways, e.g., by increasing membrane permeability, inhibiting enzyme systems, and irreversibly damaging the DNA of bacterial cells.

3.4. Bacillus cereus

Bacillus cereus (B. cereus) specimen were isolated only in the wheat and chia sprouts, because this bacterial species is more present in cereals (Rahnama et al., 2023). The results (Table 1) showed that the SS/SSP wheat sample had a significant reduction of *B. cereus* only when compared to SS/USP. Thus, it can be suggested that sanitization of both seeds and sprouts is not able to reduce the microbial load of *B. cereus* adequately. In comparison, SS/SSP chia samples showed a significant reduction of *B. cereus* only when compared to USS/USP samples, suggesting that sanitization of the seeds only or the sprouts alone is not able to reduce contamination by *B. cereus*.

It is worth noting that this microorganism is involved in outbreaks of foodborne illnesses associated with vegetable products (Choi and Kim, 2020; Glasset et al., 2016; Ultee et al., 1999) and sprouts (Portnoy et al., 1976). Foodborne illnesses caused by *B. cereus* usually occur if the product has more than 5 log CFU/g (Harmon et al.,1987). Although the number of *B. cereus* cells required to produce sufficient emetic toxin to cause diseases is still unclear, studies have shown that levels of 10^3 to 10^{10} CFU/g have been found in foods involved in cases of emetic disease, and in most cases, there were at least 10^5 CFU/g (Arnesen et al., 2007; Dietrich et al., 2021). Thus, the unsanitized wheat and chia sprouts in the present study could be a risk for the development of FBD, as they showed values greater than 5 log CFU/g. In addition, the values of *B. cereus* found in the wheat and chia sprouts in the present study are above the criteria established by the Brazilian legislation (Brasil, 2022) of 5x102 CFU/g (2.69 log CFU/g); therefore, wheat sprouts can be rated as unfit for consumption in Brazil.

Just like this study, the one by Pao et al. (2005) examined the growth of *Bacillus* spp. in sprouts grown in a domestic environment and found no growth of *B. cereus* during

germination in lentil, alfalfa, and mung bean sprouts. However, in radish and broccoli sprouts, they found values close to 5 log CFU/g. In a study conducted in natural products stores in the Washington area, *B. cereus* was found to have values ≥ 3 MPN/g in 69% of 98 seed samples tested (Harmon et al., 1987), with the average microbial load being 5.39 log CFU/g for wheat sprouts, as found in the present study.

3.5. Listeria monocytogenes

Listeria monocytogenes (*L. monocytogenes*) is a pathogenic bacterium that can be found in soil, water, and organic material and can cause a severe infection called listeriosis (Zhu et al., 2017). The severity of listeriosis can range from mild gastroenteritis to severe disease conditions (septicemia, encephalitis, meningitis, abortions, and stillbirths), resulting in a high mortality rate in immunocompromised populations (Swaminathan and Gerner-Smidt, 2007). *L. monocytogenes* accounts for 19% of total deaths caused by consumption of contaminated food in the US (Scallan et al., 2011). Owing to the risk of infection related to food consumption, limits have been established on the maximum allowable values of *L. monocytogenes* strains that can be present in these products. In Brazil, legislation states that ready-to-eat foods can have maximum values of 10^2 CFU/g or mL of *L. monocytogenes* (Brasil, 2022). Similarly, in the European Union, the legislation on contamination by *L. monocytogenes* in ready-to-eat foods also does not allow the number of this bacterium to exceed 10^2 CFU/g (European Commission, 2005).

Although *L. monocytogenes* has been related to some food outbreaks with sprouts (CDC, 2015; Garner and Katharina, 2016), this microorganism was not found in the sprouts in the present study, neither in the study by Abadias et al. (2008), who evaluated the microbiological quality of minimally processed fruits and vegetables and sprouts from retail establishments in Spain. These results were different from those reported by Seo et al. (2010), who made a microbial evaluation in 112 samples of sprouts (broccoli, alfalfa, soybean, and clover) produced in Seoul, Korea, and found the presence of this microorganism in one sample.

It is worth noting that these bacteria are difficult to isolate since microorganisms capable of growing on selective enrichment medium used for recovery of *L. monocytogenes* to the levels required for detection may be present in the food analyzed, preventing the growth of *L.*

monocytogenes through simple competition. In addition, the levels of *L. monocytogenes* in sprouts may be lower than the threshold required for direct detection and recovery, thus resulting in false negative test results (Cauchon et al., 2017).

Chlorine sanitization treatments appear to be promising in reducing *L. monocytogenes* in seeds and sprouts. Iacumin and Comi (2019) inoculated *L. monocytogenes* strains on mung bean seeds and demonstrated that the levels of *L. monocytogenes* can be reduced after 4 washes in chlorinated water solution (100 ppm) from 3.6 log CFU/g to <10 CFU in seeds, and from 9.8 log CFU/g to 1.9 log CFU/g in sprouts. In addition, Lee et al. (2002) investigated the inhibition by chemical treatment of *L. monocytogenes* inoculated into mung bean sprouts purchased from commercial stores in Washington and showed that the use of sodium hypochlorite (200 ppm) for 10 minutes was able to reduce the amount of the bacteria by 1.02 log CFU/g. In another work, treatment with aqueous chlorine dioxide (100 ppm) for 5 minutes reduced the population of *L. monocytogenes* inoculated into mung bean sprouts by 1.5 log CFU/g (Jin and Lee, 2007).

3.6. Salmonella spp

Salmonella spp. is a bacterium that causes food infection and, in some cases, it can cause death. It is transmitted through food contaminated with feces of animals such as poultry, pigs, etc. It is estimated that *Salmonella* causes about 1.35 million infections, 26,500 hospitalizations, and 420 deaths in the United States every year (CDC, 2023b). According to Miyahira et al. (2021), *Salmonella* spp was the most involved microorganism in sprout-related outbreaks from 1988 to 2020.

However, in the present work, no *Salmonella* spp strains were isolated in any of the analyzed samples, and this result is considered satisfactory according to the Brazilian legislation (Brasil, 2022) and the European commission regulation (European Commission, 2005), which determine that such strains must be absent. Similarly, Tornuk et al. (2011) investigated the presence of *Salmonella* spp. in wheat seed and sprouts, and Iacumin and Comi (2019) determined the quality of mung bean sprouts produced and sold in Italy between 2012 to 2016. Neither study found the presence of this microorganism.

However, in a trial aimed at evaluating the microbiological quality of 345 samples of minimally processed ready-to-eat vegetables, including 112 broccoli, alfalfa, soybean, and clover sprouts, Seo et al. (2010) found the presence of this microorganism in 3 samples of

sprouts. In a survey by Saroj et al. (2006) that evaluated the microbiological quality of 124 sprouts marketed in India, *Salmonella* spp. was found in 24 samples. It is worth noting that owing to the high risk of sprout-borne *Salmonella* infection, there is a need to improve the efficiency of detecting the presence of *Salmonella* spp. in sprouts, since this microorganism may be present at low concentrations or be sanitizer-injured (Zheng et al., 2015). Therefore, enrichment steps are considered as critical points in the detection of *Salmonella* spp. in sprouts, as they allow the rescue of cells of the bacterium to detectable levels (Zheng et al., 2015). Thus, in 2023, the Food and Drug Administration published an update of the Bacteriological Analytical Manual (FDA, 2023), with new standards for the analysis of *Salmonella* spp. in sprouts, indicating the use of a specific broth for microbiological analysis of sprouts, the Universal Preenrichment Broth.

3.7. Water analysis

Seeds and sprouts can be contaminated under pre- and post-harvest conditions through cross-contamination (Miyahira and Antunes, 2021). Therefore, the water used for germination and rinsing of sprouts and seeds was analyzed to assess whether cross-contamination could have occurred during germination and sanitization. No total coliforms and *E. coli* were detected in the water samples; therefore, it can be concluded that the water being used had no effect on increasing the microbial load of the study samples.

4. **Conclusions**

Although the consumption of sprouts has health benefits, one needs to be careful about its production and handling, because germination is characterized as a favorable environment for microbiological proliferation. One limitation of the present study was the small number of samples, as well as the fact that strains of *E. coli*, *L. monocytogenes* and *Salmonella* spp. were not isolated, which did not allow us to evaluate the efficacy of the treatment with sodium hypochlorite at 200ppm for elimination of these pathogenic bacteria.

Disinfection with a household sanitizer reduced the microbial load of the sprout, especially as regards total coliforms and mesophilic aerobic bacteria. However, the microbial load remained high for both of them, which may have interfered with the possible detection of *L. monocytogenes* and *Salmonella* spp. Germination in the home environment seems to be a

good strategy for inclusion of a food item with high nutritional value in the diet; therefore, it is essential that seeds suitable for germination - which have been submitted to more effective disinfection processes without changes to their germination potential - should be marketed. In this sense, the adoption of physical methods of seed sanitization seems to be a good alternative, since they can reduce the microbial load internalized in the seeds and meet the interests of some consumers, who consider the use of chemical sanitizers as contradictory to the ingestion of natural foods such as sprouts.

In addition, national competent bodies need to create and disseminate a document of good production practices focused on the cultivation of sprouts, including in domestic environment, similar to the FDA - which is focused on industrial production - so that consumers can produce and safely consume this food at home from the microbiological point of view.

Finally, further studies should be conducted to develop new sanitization techniques in the home environment that are effective in reducing the microbial load of sprouts as well as other fresh foods.

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CRediT authorship contribution statement

Vanessa dos Santos Chiappetta Nogueira Salgado: Formal analysis, Writing – original draft, Investigation. **Flávia Oliveira Brito**: Formal analysis, Writing – original draft, Investigation. **Paula Stephany de Melo Rodrigues**: Formal analysis, Writing – original draft, Investigation. **Adriane Elisabete Costa Antunes**: Writing – review & editing, Investigation. **Raquel Gouvêa**: Methodology, Formal analysis. **Lilia Zago**: Methodology, Formal analysis, Writing – review & editing, Investigation. **Roberta Fontanive Miyahira**: Conceptualization, Methodology, Formal analysis, Funding acquisition, Investigation, Writing – review & editing, Supervision.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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8 **CONSIDERAÇÕES FINAIS**

Este estudo ressaltou a importância da germinação das sementes de chia como um processo eficiente para aprimorar suas propriedades nutricionais. Durante a germinação, ocorreu um aumento significativo nas proteínas, assim como a preservação da qualidade lipídica do broto em comparação com a semente. Além disso, a germinação também resultou em um aumento no potencial antioxidante das sementes de chia.

Entretanto, o estudo alerta para a preocupação com a qualidade microbiológica das sementes germinadas. O alto teor de coliformes totais e aeróbios mesófilos, bem como a presença de *Bacillus cereus* mesmo após a sanitização com hipoclorito de sódio, destaca a necessidade de medidas adicionais para garantir a segurança microbiológica dos brotos de chia antes do consumo.

Portanto, é essencial que órgãos regulatórios desenvolvam e divulguem diretrizes de boas práticas de produção para o cultivo seguro de brotos de chia, especialmente em ambientes domésticos. Essas medidas visam incentivar a produção segura dos brotos, reduzindo os riscos de doenças transmitidas por alimentos associadas ao seu consumo cru e não tratado termicamente.

Em resumo, a germinação das sementes de chia pode ser uma estratégia benéfica para melhorar suas propriedades nutricionais e antioxidantes. No entanto, a segurança microbiológica dos brotos de chia é uma questão importante que precisa ser abordada com cuidado para garantir que esses alimentos sejam seguros para o consumo humano.

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