UNIVERSIDADE FEDERAL DE VIÇOSA

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EFEITO DA HIDRATAÇÃO ASSISTIDA POR ULTRASSOM DE FORMA INTERMITENTE NA SEMENTE DE ABÓBORA: IMPACTO NA CINÉTICA DE HIDRATAÇÃO, GERMINAÇÃO E QUALIDADE DA FARINHA GERMINADA

VIÇOSA – MINAS GERAIS

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Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, para obtenção do título de Master Scientiae.

Orientador: Dr. Bruno Ricardo de Castro Leite Júnior

Coorientadores: Dra. Alline Artigiani Lima Tribst, Dr. Paulo Henrique Costa Paiva

VIÇOSA - MINAS GERAIS

2024

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T

P116e
2024

Pacheco, Flaviana Coelho, 1993-
Efeito da hidratação-assistida por ultrassom de forma intermitente na semente de abóbora: impacto na cinética de hidratação, germinação e qualidade da farinha germinada / Flaviana Coelho Pacheco. – Viçosa, MG, 2024.
1 dissertação eletrônica (134 f.): il. (algumas color.).

Texto em português e inglês.
Orientador: Bruno Ricardo de Castro Leite Júnior.
Dissertação (mestrado) - Universidade Federal de Viçosa, Departamento de Ciência e Tecnologia de Alimentos, 2024.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2024.688
Modo de acesso: World Wide Web.

1. Abóbora - Semente. 2. Tecnologia de alimentos. 3. Germinação. 4. Hidratação. 5. Valor nutritivo. 6. Sonicação. I. Leite Júnior, Bruno Ricardo de Castro, 1989-. II. Universidade Federal de Viçosa. Departamento de Ciência e Tecnologia de Alimentos. Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos. III. Título.

CDD 22. ed. 664.3

Bibliotecário(a) responsável: Bruna Silva CRB-6/2552

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APROVADA: 22/07/2024

ASSENTIMENTO:

Documento assinado digitalmente
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Verifique em https://validar.iti.gov.br

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Data: 09/10/2024 16:45:15-0300
Verifique em https://validar.iti.gov.br

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Orientador

AGRADECIMENTOS

A Deus pela oportunidade concedida, por ter me guiado até aqui e por abençoar abundantemente o meu caminho.

Aos meus pais, Regina e João Carlos, pelo apoio incondicional, amor, dedicação e incentivo nesta etapa. Agradeço por não medirem esforços para que eu realize os meus sonhos.

Aos meus irmãos, Ana Flávia e Jean Carlos, por sempre estarem ao meu lado, pela amizade, apoio e paciência. Vocês são tudo para mim, amo muito vocês.

À Universidade Federal de Viçosa, pela oportunidade de ampliar meus conhecimentos em uma excelente instituição.

À Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) pela concessão da bolsa de estudos e pelo financiamento do projeto (APQ- 00785-23).

Ao meu orientador, Professor Dr. Bruno Ricardo de Castro Leite Júnior, pelos ensinamentos, apoio, diálogos, incentivo e confiança durante todos esses anos de orientação.

Aos meus coorientadores, Dra. Alline Artigiani Lima Tribst e Professor Dr. Paulo Henrique Costa Paiva, pela participação na banca de defesa, ensinamentos, colaboração e assistência na realização desse trabalho.

Ao Pedro Esteves Duarte Augusto pela participação na banca de defesa, pela disponibilidade e contribuições tão importantes e necessárias.

Aos integrantes do LIPA, em especial Jeferson, Fábio e Irene, por serem ótimos colegas de trabalho, pela cooperação, conversas, cafezinhos e boas risadas.

Ao Mateus, meu companheiro, que sempre está ao meu lado, me dando confiança, carinho, paciência, alegria e amor. ÀCarol, minha cunhada, pela amizade e carinho.

Agradeço a todas as pessoas que, de alguma forma, fizeram parte dessa conquista e contribuíram para o meu crescimento pessoal e profissional.

BIOGRAFIA

Flaviana Coelho, Pacheco, filha de João Carlos Pacheco e Regina Célia Coelho Pacheco, nasceu em São João Nepomuceno, Minas Gerais, em 07 de outubro de 1993.

Em março de 2016, ingressou no curso de Química Bacharel na Universidade Federal de Viçosa (UFV), sendo interrompido em julho de 2018. Iniciando o curso de Engenharia de Alimentos na Universidade Federal de Viçosa (UFV) em 2018, graduando-se em julho de 2022.

Em setembro de 2022 iniciou o mestrado no Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, na Universidade Federal de Viçosa, submetendo-se à defesa de dissertação em julho de 2024.

RESUMO

PACHECO, Flaviana Coelho, M.Sc., Universidade Federal de Viçosa, julho de 2024. **Efeito da hidratação-assistida por ultrassom de forma intermitente na semente de abóbora: Impacto na cinética de hidratação, germinação e qualidade da farinha germinada.** Orientador: Bruno Ricardo de Castro Leite Júnior. Coorientadores: Alline Artigiani Lima Tribst e Paulo Henrique Costa Paiva.

A semente de abóbora (*Cucurbita moschata*) tem se destacado como subproduto de potencial aproveitamento industrial devido ao seu alto valor nutricional, especialmente pelas altas concentrações de proteínas e lipídios, e pela presença de compostos bioativos, como fenólicos totais e outros compostos com capacidade antioxidante. Apesar deste potencial, a utilização destas sementes na alimentação humana é relativamente baixa, sendo necessário o desenvolvimento estudos para sua incorporação no setor alimentício. Técnicas de bioprocessamento, como a germinação, podem melhorar as qualidades nutricionais e funcionais da semente, tornando-as mais atrativas. No entanto, antes da germinação, as sementes devem ser hidratadas, um processo longo e caro, sendo de grande interesse sua otimização. Para superar essas desvantagens, tecnologias não convencionais, como o ultrassom (US), têm sido estudadas para reduzir o tempo de hidratação de grãos e sementes. O estudo foi dividido em dois capítulos, o primeiro investigou a cinética de hidratação assistida por ultrassom (US) de forma intermitente (1–4 ciclos de 10 min, 38 W/L, 25 kHz, a 25 ou 45 °C) de sementes de abóbora e seu impacto na cinética de germinação, bem como na qualidade nutricional e biológica das farinhas germinadas. No segundo capítulo foi avaliado o efeito do ultrassom considerando as melhores condições de tratamento (US-20min aplicado à 25 °C e 45 °C) na qualidade nutricional, física, tecno-funcional e estrutural da farinha otimizada. A sonicação reduziu o tempo de hidratação (em até 54,3%), diminuiu a fase de latência da germinação (em até 19,3%) e aumentou a capacidade máxima de germinação (em até 5%) ($p < 0,05$). Além disso, o tratamento ultrassônico levou a um maior comprimento das radículas (em até 19,4%) após a germinação. Em relação à qualidade da farinha, o ultrassom aumentou o teor de proteína e lipídios (≤ 23% e ≤ 8,4%, respectivamente), com o melhor resultado observado com US aplicado em 4 ciclos de 10 min a 45 °C. Além disso, amostras sonicadas por 2 ciclos de 10 min apresentaram níveis mais altos de açúcares redutores (≤ 79%), compostos fenólicos (≤ 81%) e proteína solúvel (≤ 19,8%) em comparação à farinha germinada não tratada com US. Já avaliando as condições otimizadas da farinha a hidratação assistida por US resultou em uma farinha mais leve e uma estrutura mais porosa e dispersa, que foi intensificada pelo aumento da temperatura de hidratação. A germinação promoveu alterações nos hidrocarbonetos alifáticos, aminoácidos e carboidratos da farinha, especialmente nas ligações C-H e C-O, conforme evidenciado por FTIR. Além disso, a germinação e a sonicação diminuíram o grau de cristalinidade do amido (≤14,3). O tratamento por US não afetou o perfil mineral nem de ácidos graxos das farinhas. Por outro lado, a sonicação foi eficiente em aumentar o conteúdo de GABA (15,6% e 13,7%), ABTS (29,4% e 9,7%) e atividades antioxidantes DPPH (40,8% e 2,4%) em comparação com farinhas não sonicadas a 25 °C e 45 °C, respectivamente. Em relação às propriedades tecno-funcionais, o US aumentou a capacidade de retenção de água, índice de solubilidade, capacidade de espuma e estabilidade da espuma em até 49%, 68%, 143% e 208%, respectivamente. Os efeitos positivos da sonicação, particularmente a 25 °C, nas propriedades nutricionais e tecno-funcionais da farinha de semente de abóbora germinada destacam seu potencial como uma alternativa sustentável para o desenvolvimento de ingredientes alimentícios inovadores, com possíveis aplicações em produtos como pães, bolos, biscoitos e bebidas.

Palavras-chave: Tecnologias emergentes; Germinação; Hidratação; Qualidade nutricional; Sementes de abóbora; Sonicação.

ABSTRACT

PACHECO, Flaviana Coelho, M.Sc., Federal University of Viçosa, July 2024. **Effect of intermittent ultrasound-assisted hydration on pumpkin seed: Impact on hydration kinetics, germination and quality of germinated flour.** Advisor: Bruno Ricardo de Castro Leite Júnior. Co-supervisors: Alline Artigiani Lima Tribst and Paulo Henrique Costa Paiva.

Pumpkin seeds (Cucurbita moschata) have stood out as a byproduct with potential industrial use due to their high nutritional value, especially due to their high concentrations of proteins and lipids and the presence of bioactive compounds, such as total phenolics and other compounds with antioxidant capacity. Despite this potential, the use of these seeds in human nutrition is relatively low, and studies are needed to incorporate them into the food sector. Bioprocessing techniques, such as germination, can improve the nutritional and functional qualities of the seed, making them more attractive. However, the seeds must be hydrated before germination, a long and expensive process, and its optimization is of great interest. To overcome these disadvantages, unconventional technologies, such as ultrasound (US), have been studied to reduce the hydration time of grains and seeds. The study was divided into two chapters. The first investigated the kinetics of intermittent ultrasound-assisted (US) hydration (1–4 cycles of 10 min, 38 W/L, 25 kHz, at 25 or 45 °C) of pumpkin seeds and its impact on germination kinetics, as well as on the nutritional and biological quality of the germinated flours. The second chapter evaluated the effect of ultrasound considering the best treatment conditions (US-20min applied at 25 °C and 45 °C) on the optimized flour's nutritional, physical, techno-functional, and structural quality. Sonication reduced hydration time (by up to 54.3%), decreased the germination lag phase (by up to 19.3%), and increased maximum germination capacity (by up to 5%) ($p < 0.05$). Furthermore, ultrasonic treatment led to greater radicle length (up to 19.4%) after germination. Regarding flour quality, ultrasound increased the protein and lipid content (≤ 23% and ≤ 8.4%, respectively), with the best result observed with the US applied in 4 cycles of 10 min at 45 °C. Furthermore, samples sonicated for 2 cycles of 10 min showed higher levels of reducing sugars (≤ 79%), phenolic compounds (≤ 81%), and soluble protein (≤ 19.8%) compared to germinated flour not treated with US. Evaluating the optimized flour conditions, US-assisted hydration resulted in lighter flour and a more porous and dispersed structure, intensified by increasing the hydration temperature. Germination promoted changes in the aliphatic hydrocarbons, amino acids, and carbohydrates of the flour, especially in the C-H and C-O bonds, as evidenced by FTIR. Furthermore, germination and sonication decreased the degree of starch crystallinity (≤14.3). US treatment did not affect the flour's mineral or fatty acid profile. On the other hand, sonication was efficient in increasing the content of GABA (15.6% and 13.7%), ABTS (29.4% and 9.7%), and DPPH antioxidant activities (40.8% and 2.4%) compared to non-sonicated flours at 25 °C and 45 °C, respectively. Regarding the techno-functional properties, the US increased the water retention capacity, solubility index, foaming capacity, and foam stability by up to 49%, 68%, 143%, and 208%, respectively. The positive effects of sonication, particularly at 25 °C, on the nutritional and techno-functional properties of sprouted pumpkin seed flour highlight its potential as a sustainable alternative for the development of innovative food ingredients, with possible applications in products such as bread, cakes, and cookies, and beverages.

Keywords: Emerging technologies; Germination; Hydration; Nutritional quality; Pumpkin seeds; Sonication.

Sumário

INTRODUÇÃO GERAL

A abóbora (*Cucurbita moschata*) é uma cultura de grande importância comercial, cultivada mundialmente (Li et al., 2021). As sementes de abóbora são frequentemente descartadas como resíduos ou utilizadas na alimentação animal, sendo pouco aproveitadas para o consumo humano (Pacheco et al., 2023). No entanto, essas sementes são ricas em diversos componentes (Dotto & Chacha, 2020), como proteínas, lipídios, compostos bioativos e ácido γ-aminobutírico (GABA) (Liang et al., 2022).

Normalmente, a digestibilidade das proteínas vegetais é baixa. Além disso, a quantidade de GABA presente naturalmente nas plantas é insuficiente para atender às necessidades nutricionais como alimento funcional (Wu & Shah, 2018). Consequentemente, métodos bioquímicos, como a germinação, têm sido aplicados para melhorar a biodisponibilidade desses compostos, bem como de outros como fenólicos e antioxidantes (Ding et al., 2018).

Diversos estudos demonstraram que a germinação é um método econômico e eficaz para favorecer a formação de compostos bioativos em sementes de plantas (Ding et al., 2018). No entanto, esse processo pode ser influenciado por diversos fatores como hidratação, temperatura, luz e oxigênio (Kalita et al., 2021). Dentre esses, a hidratação é de grande importância para a quebra inicial da dormência, processo que inicia a germinação (Louf et al., 2018). Embora seja um processo simples de imersão em água, a hidratação é marcada por ser uma etapa longa e onerosa, que requer condições específicas e controladas (Kalita et al., 2021). Consequentemente, acelerar esse processo é muito desejável, especialmente do ponto de vista industrial.

O ultrassom (US) tem se mostrado eficaz na redução do tempo de hidratação e germinação das sementes, além de melhorar as propriedades da farinha obtida (Miano & Augusto, 2018). Estudos demonstram que o ultrassom pode aumentar a taxa de absorção de água em feijão comum (Miano et al., 2018), grãos de trigo (Guimarães et al., 2020), trigo duro (Yıldırım, 2022) e arroz (Aires et al., 2023). Além disso, observou-se impacto positivo na germinação de grãos de feijão-azuki (Chiu, 2021) e sementes de arroz (Huang et al., 2024). O uso do ultrassom também resultou em aumento no teor de proteínas e lipídeos (Pacheco et al., 2024), GABA, compostos bioativos (Banura & Singh, 2023), entre outros.

Apesar da existência de estudos que mostram que o ultrassom apresenta resultados satisfatórios em diversos processos de germinação, como abordado no Capítulo 1, os efeitos da aplicação desta tecnologia na semente de abóbora ainda não haviam sido elucidados. Portanto, este projeto propôs investigar a aplicação da tecnologia de ultrassom visando avaliar a cinética de hidratação de sementes de abóbora assistida. Especificamente no capítulo 2, o ultrassom (38 W/L e 25 kHz), foi aplicado durante a hidratação das sementes de abóbora de forma intermitente em diferentes tempos (até 4 ciclos de 10 min) e temperaturas (25 e 45 °C). Além disso, este estudo também investigou o impacto nas propriedades de germinação das sementes e nas propriedades nutricionais e biológicas das farinhas obtidas, com o objetivo de determinar as melhores condições de processo. Desta forma, o Capítulo 3 abordou o impacto do ultrassom durante a hidratação das sementes de abóboras sob as condições de processos otimizadas (2 ciclos de 10 min a 25 °C e 45 °C) na qualidade nutricional, física, biológicas, tecno-funcionais e estruturais da farinha resultante. A partir destes resultados, espera-se diversificar as aplicações do ultrassom na hidratação de sementes e ampliar a utilização deste subproduto permitindo a viabilização econômica em termos de qualidade, custo e rendimento.

Chapter 1: ADVANCES IN ULTRASOUND-ASSISTED SEED HYDRATION: POTENTIAL TO IMPROVE GERMINATION AND FLOUR QUALITY

Abstract

Seed germination is an interesting strategy to improve nutrient bioavailability and reduce antinutrients, producing more nutritious and healthy food ingredients. However, ensuring optimal conditions for germination, such as hydration time and temperature, is crucial to break dormancy and ensure the success of the germination process. In addition, this process presents several challenges because it is slow and expensive, increasing the risks of microbiological contamination. To overcome these limitations, ultrasound (US) has been explored as a promising technique to improve the germination of different traditional and emerging raw materials. However, its impact on the entire germination process and flour quality still needs to be determined. This review analyzed the effect of ultrasound on seed germination, highlighting its influence on hydration, germination, and quality of the resulting flours.

Keywords: Bioavailability of nutrients, emerging technologies, food quality, added value, sustainability.

1. Introduction

Seeds have significant economic relevance, being cultivated globally as vital sources of essential nutrients, including proteins, fibers, lipids, vitamins, minerals, and bioactive compounds of industrial interest (Tieri et al., 2020; Garutti et al., 2022). However, these foods also have nutritional limitations and anti-nutritional factors, such as phytate, tannins, and amylase inhibitors, which can impair their digestibility and absorption (Samtiya et al., 2020).

Germination is a promising strategy to overcome these limitations, inducing biochemical and physiological changes that increase nutrient bioavailability, reduce antinutrients and allergens, and enhance sensory characteristics (Andressa et al., 2023; Liu et al., 2022). To ensure effective germination, it is essential to consider favorable conditions such as seed hydration (Rifna et al., 2019). Hydration, the first germination stage, involves increasing moisture by soaking the seeds in water. However, achieving equilibrium humidity can require a prolonged time (up to 36 hours), high energy costs, and considerable amounts of water, depending on the type of seed, in addition to being a process with a high risk of contamination and microbial growth (Carvalho et al., 2021). Therefore, optimizing this process is crucial (Miano & Augusto, 2018b; Jensen et al., 2023).

Ultrasound (US) is a technology that has been explored to improve seed germination, however, its impact during the process steps (hydration and incubation) needs to be elucidated (Miano et al., 2016). Its application at different stages of germination, such as before, during, or after hydration, combined with variations in time, temperature, power, frequency, form of application (intermittent and continuous pulses), and type of equipment (probe or bath), can result in different effects on the cellular structure of food during and after germination (Miano et al., 2018; Guimarães et al., 2020; Kalita et al., 2021).

The observed improvements in ultrasound-assisted germination represent a significant opportunity in the food industry, both to increase crop production and to develop new nutritious and innovative food products. This potential of ultrasound treatment is inspiring, but there are gaps and challenges that need to be addressed in order to fully understand its mechanisms and optimize its applications, such as: treatment parameters, species-specific effects, potential harm, interaction with other factors, standardization of methods, and application on a commercial scale. Bibliometric analysis is a statistical tool for obtaining consistent data from the literature that describes the main sources of interest (Melo et al., 2021) and become an interesting option to highlight the evolution of perspectives, challenges, and future trends in scientific research.

Therefore, this review seeks to contribute to this understanding by evaluating the use of ultrasound treatment in seed hydration, scientific development trends and possible applications. Understanding the mechanisms involved in germination is crucial for our research and development of new products, as the population seeks alternative products that offer health benefits.

2. Methodology

2.1. Data collection

The present study was based on a narrative review of the literature. Data was collected in the Scopus and Web of Science Core Collection databases with the following search equation: (germination OR germinated OR sprouting) AND ultrasound in the filter "Search within Article title, Abstract, Keywords." The documents were selected in a timeless way and filtered by research articles.

2.2. Bibliometric analysis

The articles from the search in the databases were exported in BibTeX format with all their respective information. Subsequently, the files of both databases were compiled in RStudio Software (Desktop version 2022.12.0+353, RStudio, Inc., Boston, USA) with Bibliometrix package for R (version 4.1.3, R Foundation for Statistical Computing, Vienna, Austria) to remove duplicate jobs between the databases. The selected articles were then grouped into a single file and exported in CVS and Exel format.

RStudio was instrumental in analyzing citations and the number of publications over time, as well as in identifying publications, cooperation between countries, and the thematic map of authors' keywords. In addition, we employed VOSviewer (version 1.6.19) specifically for assessing the co-occurrence of keywords and Microsoft Excel

2019 (Microsoft, Redmond, WA, USA) for analyzing the annual volume of publications and the number of citations by country.

3. Bibliometric analysis

The initial search of the present study returned 636 documents, 314 from the Scopus and 322 from the WOS. Of these, 133 were duplicates, which resulted in 503 articles analyzed. A total of 1935 authors were detected, with an average of 5.46 per document and an annual growth rate of 5.15%. The first article on the use of ultrasound in germination occurred in 1950 (Figure 1A) and presented few publications until 2015 when there was an exponential growth in the number of scientific articles. This increase in recent years can be attributed mainly to industry and research interest in applying whole seeds in food matrices, either as an ingredient or as a raw material. In this context, the present study preferentially reviewed studies published between 2015 and 2024. In addition, 1536 keywords of the authors were detected, 28 of which had an occurrence equal to or greater than 5, as shown in Figure 1B. Co-occurrence analysis is used to identify the most relevant terms that can be used by researchers who wish to find appropriate sources related to the article. The dominant keywords are germination and ultrasonic, which are the article's main focus. In addition, other important keywords that are also highlighted are γ-aminobutyric acid (GABA)and phenolic compounds; these biological compounds have been associated with several health benefits, highlighting their importance when evaluating the nutritional quality of sprouted flours.

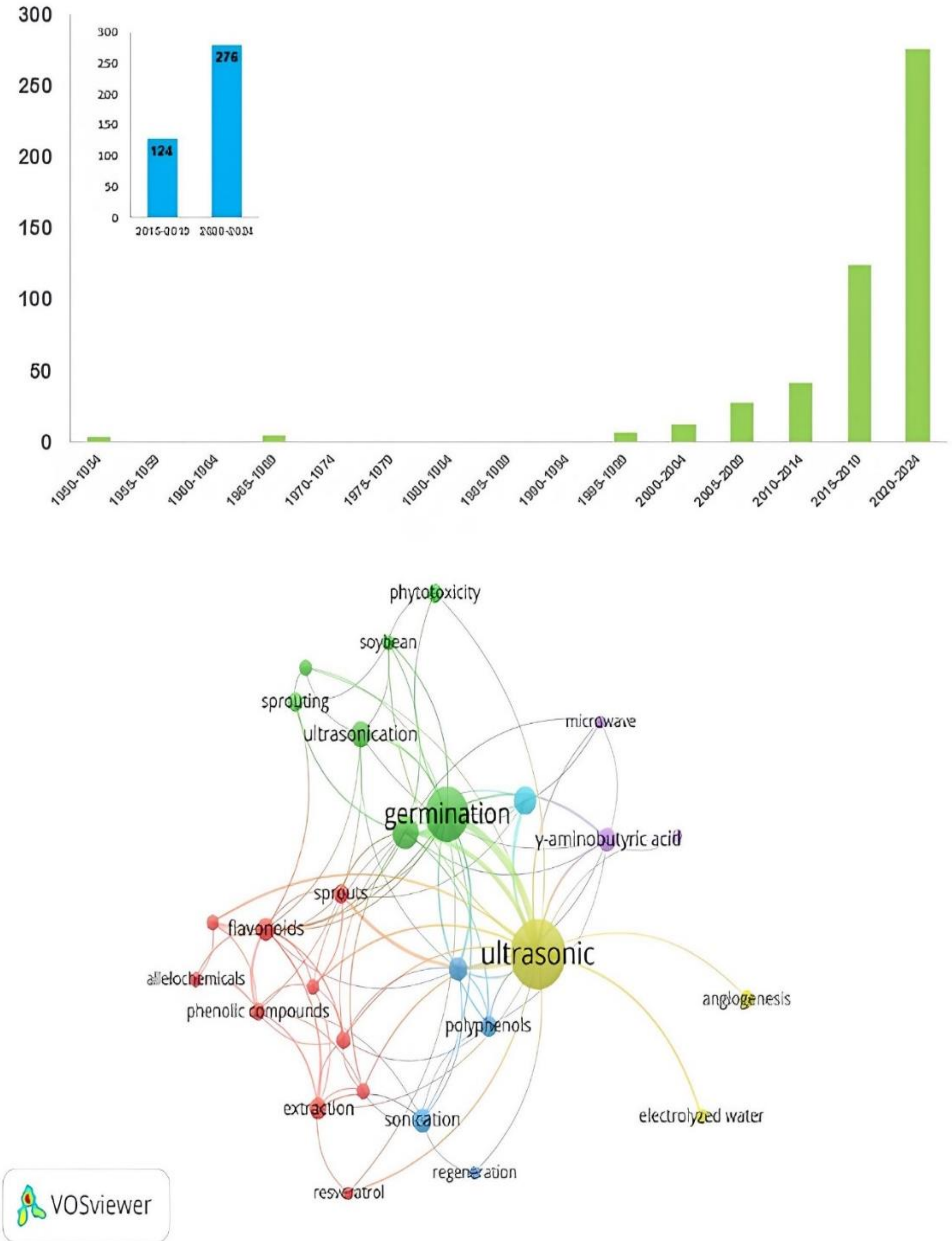

Figure 1. Publications related to germination from 1950 to 2024 (A) and occurrence of keywords (B).

4. Literature Review
4.1. Germination

Germination is a biological process that causes several changes in seeds, affecting their proximate composition and biological and structural quality (Figure 2) (Liu et al., 2022). Proteolytic enzymes degrade proteins into peptides and free amino acids, increasing digestibility and nutritional quality (Bera et al., 2023). Starch is hydrolyzed into glucose and maltose, dietary fiber becomes more soluble, and lipids break into free fatty acids, improving digestibility (El-Safy & Salem, 2013). Germination also increases levels of B vitamins, vitamin C, and carotenoids, reduces anti-nutritional compounds like phytates, and increases the bioavailability of minerals (Darwish et al., 2021). Bioactive compounds such as polyphenols and flavonoids also increase (Bera et al., 2023).

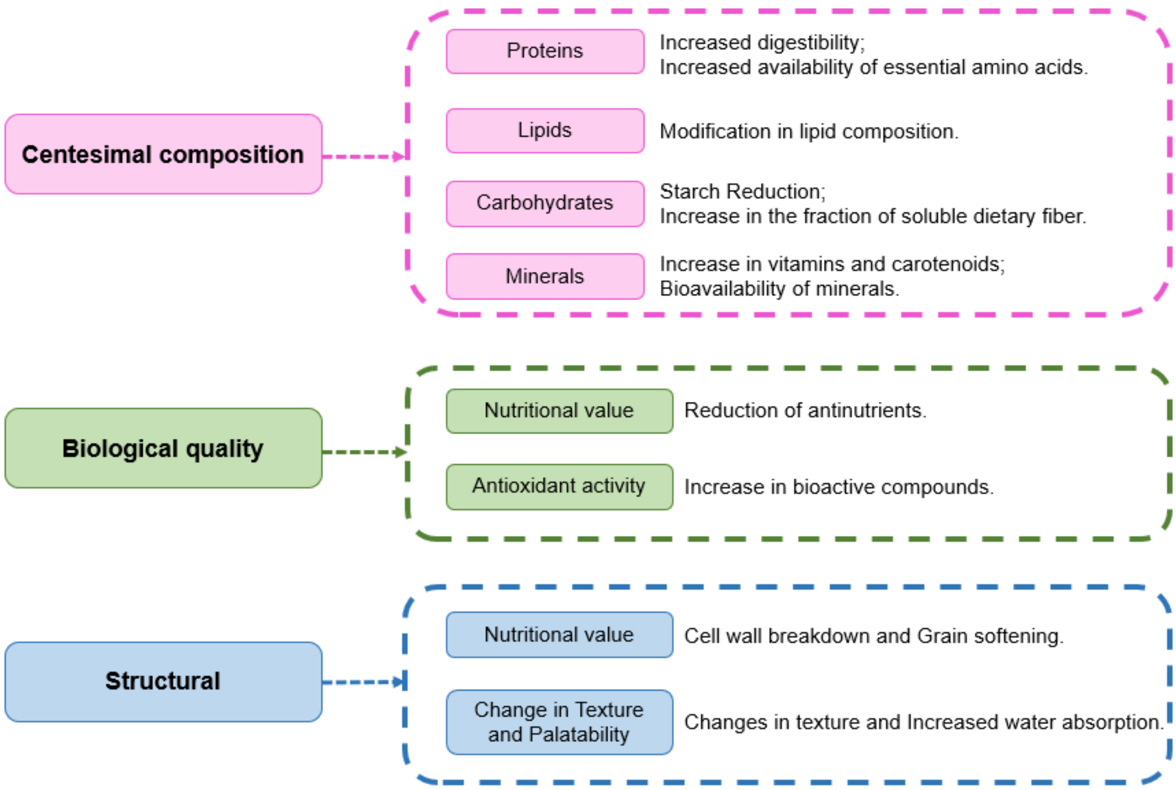

Figure 2. Main changes that occur during the germination process.

The stages of germination are divided into three stages: (i) Hydration; (ii) Preparation phase; and (iii) Growth stage (Figure 3).

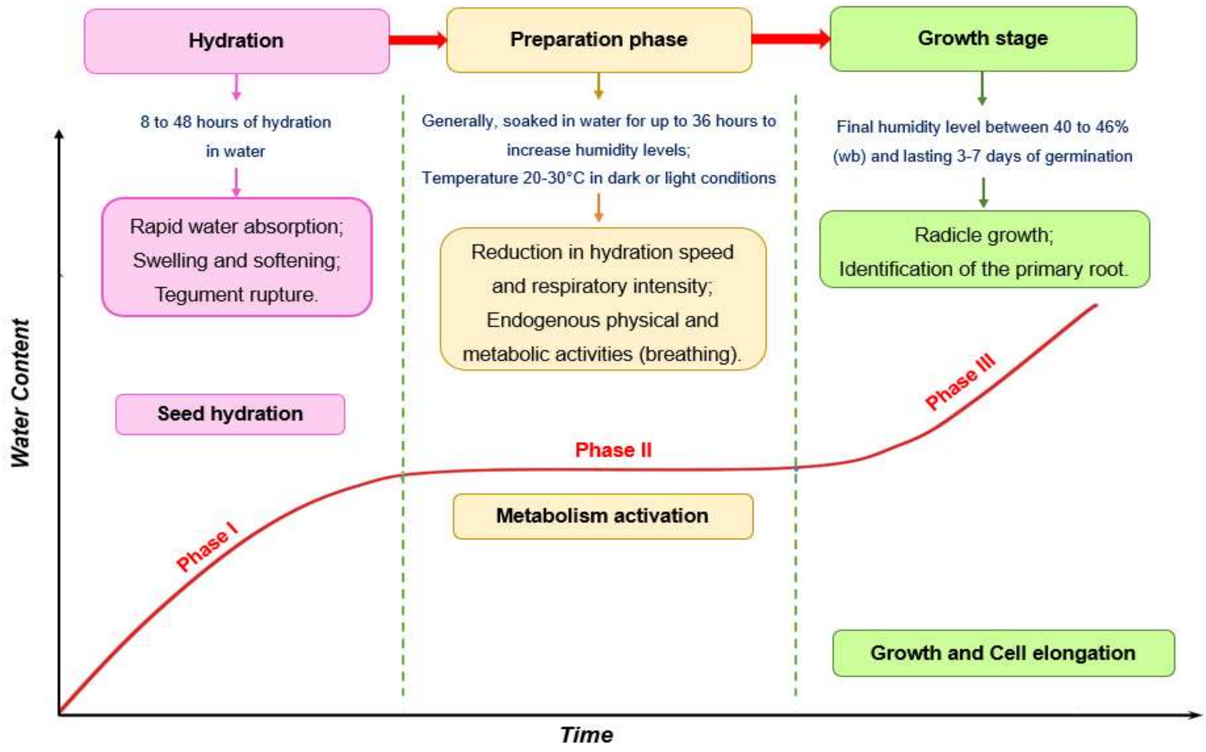

Figure 3. Stages of germination. Adapted from Bewley and Black (1978).

During phase I, a rapid absorption of water occurs due to the low water potential of the seed to achieve an adequate moisture content to activate its metabolism. This phase leads to swelling, softening, and rupture of the integument, making the grain and seed more permeable to substances (Xue et al., 2021). In stage II, the hydration process becomes limited, and the seed enzymes break down the reserve molecules into simple molecules to be used by the embryo (Al-Hazmi & Naguib, 2022). In phase III occurs the end of germination, with the rupture of the cover layers, allowing radicle growth and again increasing moisture to equilibrium (40-46%) (Tuan et al., 2019).

It is important to note that seeds may be in a physiological state called dormancy before the germination process begins. Even under ideal conditions, the seed will not germinate. To start germination, the seed must be placed in environmental conditions conducive to growth, including water, adequate temperature, oxygen, light, and relative humidity. Among these factors, water plays a pivotal role as the main regulator of germination, as the process initiates with seed hydration.

4.2. Seed hydration

Hydration is mediated by numerous processes involving enzymatic reactions and mobilization of seed storage reserves, including lipids, carbohydrates, and proteins (Nautiyal et al., 2023). Water plays a crucial role in the osmotic regulation of cells, being necessary to activate enzymes that break down starch and protein stocks into simpler compounds such as sugars and amino acids (Szczerba et al., 2021). In addition, it allows seed cells to expand, including softening, facilitating the rupture of the integument, and initiating radicle growth (Nautiyal et al., 2023).

In conventional hydration, seeds are hydrated in water for up to 36 hours to increase moisture levels to 40 to 45% (w.b.). After this process, seeds take approximately 3 to 7 days to germinate (Carvalho et al., 2021). Optimizing these steps is desirable given the long process time, costs, and risks involved.

Some studies used high temperatures during the hydration stage to accelerates this process (Mercier et al., 2015; Montanuci et al., 2015). The role of temperature in seed germination is crucial in influencing seed dormancy and germination time (Vicente et al., 2020). The temperature can be controlled during hydration (stage I) or seed incubation (stages II and III).

During stage I, temperature directly affects water uptake by seeds, influencing the rate and efficiency of hydration (Vicente et al., 2020; Kalita et al., 2021). Higher temperatures generally accelerate water absorption (Haj Sghaier et al., 2022) because water moves and penetrates faster (Miano & Augusto, 2018).

In addition, another important factor during germination is the germination power and growth yield of the seeds, which several factors can influence. Among them are hydration, previously discussed in conjunction with temperature, and the control of relative humidity and temperature during incubation in stages II and III of germination (Footitt et al., 2013).

Stages II and III of germination are sensitive to temperature, which can negatively or positively influence germination rate and radicle development (Footitt et al., 2013) it is possible to highlight three ranges of cardinal temperatures during the incubation of grains and seeds: minimum, optimal, and maximum (Figure 4).

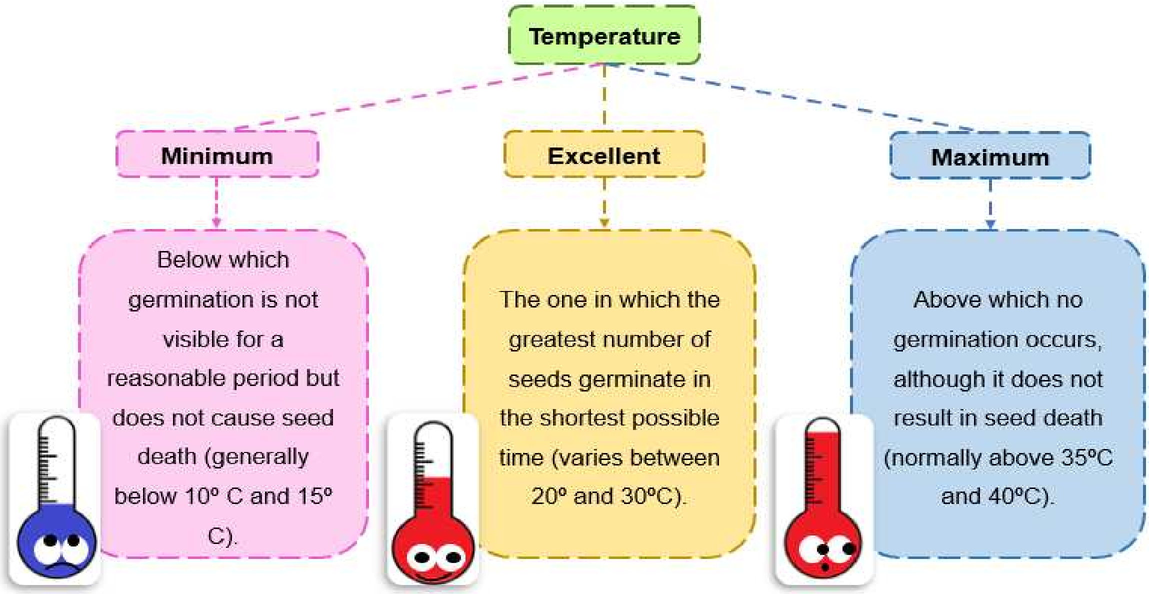

Figure 4. Cardinal temperature ranges during the germination process in phases I, II and III.

The germination at optimal temperature range is critical for a successful process. Temperatures outside this range, whether lower or higher, significantly reduce the speed of germination. This prolongs the period in which seeds are exposed to adverse environmental factors, leading to a considerable reduction in the total percentage of seeds germinating (Carvalho & Nakagawa, 2000).

As a result, in recent years, unconventional technologies have been the subject of research to accelerate hydration kinetics (Ding et al., 2018; Refna et al., 2019). These technologies include high-pressure processing, pulsed electric field, ultrasound, ozone, ultraviolet, magnetic field, microwave radiation, cold plasma, etc. In this scenario, ultrasound has been achieving greater prominence recently due to its positive impact of on phases I, II, and III of germination (Miano et al., 2018; Miano et al., 2019; Guimarães et al., 2020; Aires et al., 2023; Chiu, 2021; Zhang et al., 2023; Huang et al., 2024).

4.3. Ultrasound Technology (US): Impact on Hydration, Germination and Properties of Sprouted Flour

Ultrasound is a sustainable and environmentally friendly non-thermal technology that involves the formation of sound waves at frequencies above human hearing capacity (>20 kHz) (Pacheco et al., 2023a). Currently, there is two main types of ultrasonic equipment used in laboratories: bath ultrasound and probe ultrasound (Figure 5) (Lukić et al., 2019).

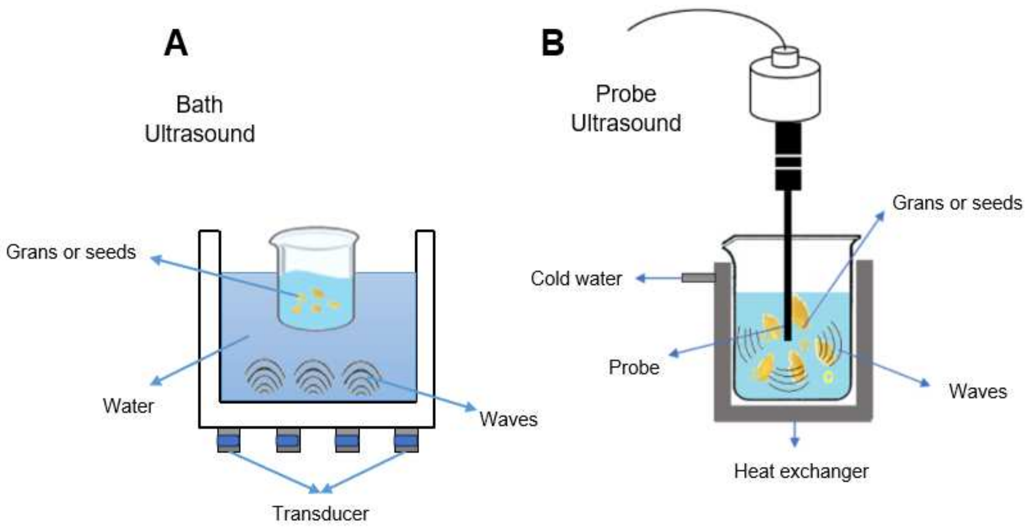

Figure 5. Bath ultrasound and probe ultrasound.

Bath ultrasound consists in a tank filled with a liquid (usually a water, in which samples are submerged), a timer button, a heater exchanger, and at least one ultrasonic transducer. This equipment emits ultrasonic waves through the liquid, creating microbubbles (cavitations) in the samples (Fu et al., 2019). Probe ultrasound, on the other hand, uses a sonication probe directly inserted into the sample, providing a more focused and powerful effect (Zhang et al., 2023).

This technology has wide applications in food mass transfer processes, such as drying, extraction, osmotic dehydration, desalination, and hydration. The generation of expansion and compression phases, and the induction of cavitation bubbles in a liquid medium when a high acoustic force is applied, are key features that make ultrasound a valuable asset in food processing methods.

Ultrasound in the seed's hydration process can improve the permeability of the seeds shell, improving its water absorption (Silva & Villela, 2011). In addition, sonication improves dormancy break and stimulates biochemical processes that prepare seeds for germination (Figure 6) (Wong et al., 2019).

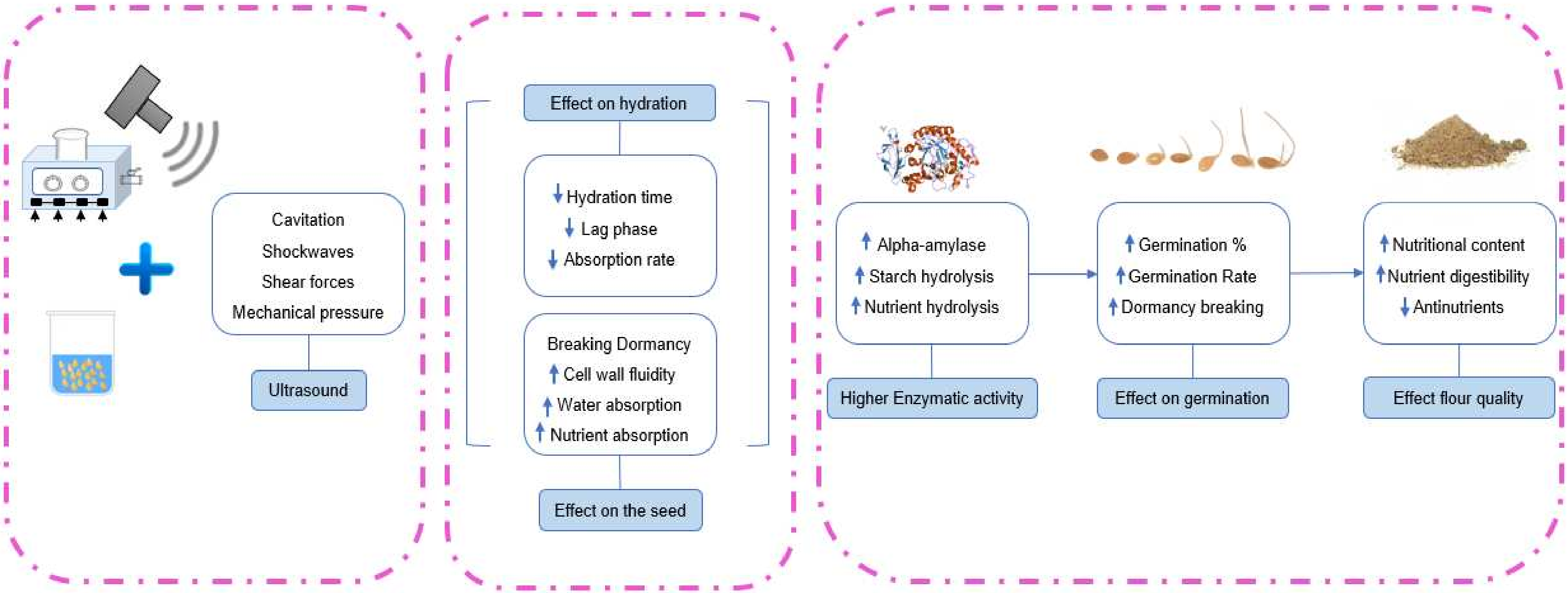

Figure 6. Impact of ultrasound on hydration, germination and quality of sprouted flour.

4.3.1. Sonication Impact on germination stage I (hydration)

Understanding the water absorption in seeds during hydration is crucial because it affects subsequent processing operations and the quality of the final product (Ulloa et al., 2015). Table 1 shows the results of studies involving the application of ultrasound to enhance the hydration process of different seeds and highlights that the process is strongly influenced by numerous variables, such as sonication power, time, temperature, type of equipment, and seed variety (Miano & Augusto, 2018b).

Generally, grains hydration time has been significantly reduced using ultrasound. Studies showed hydration time reductions of 58.8% for beans (Ulloa et al., 2015), 40% for sorghum (Patero & Augusto, 2015), 25% for mung beans (Miano et al., 2016), 35% for corn (Miano et al., 2017), 50% for rice (Kalita et al., 2024), and 38.01% for barley (Carvalho et al., 2024) with the use of bath ultrasound. In the case of probe ultrasound, a reduction of 40% was observed for wheat (Guimarães et al., 2020) and 90% for chickpeas (Bender et al., 2024).

Ulloa et al. (2015) and Miano et al. (2016) used various beans and different sonication approaches to improve their hydration. Ulloa et al. (2015) applied ultrasound for 30 minutes followed by conventional hydration, resulting in a 58.8%- hydration time reduction. On the other hand, Miano et al. (2016) applied sonication along the hydration step (10-hour process, time required to reach the equilibrium humidity), resulting in a 25% reduction. The results indicate that pretreatment with ultrasound followed by conventional hydration wasmore efficient, possibly due to the initial opening of the seed pores. Meanwhile, prolonged exposure to ultrasound can quickly saturate the seeds, limiting further water absorption.

Carvalho et al. (2024), Miano et al. (2017) and Paterno and Augusto (2015) used volumetric powers of 91 W/L, 41 W/L, and 28 W/L, resulting in a reduction in hydration time by 38%, 35%, and 40%, respectively. These results highlights that the lowest volumetric power resulted in the greatest decrease in hydration time. This suggests that even at lower potencies, ultrasonic energy intensity may be sufficient to promote a more effective effect on grain hydration (Patero & Augusto, 2015).

Temperature is another factor that can influence the results. Aires et al. (2023) varying from 35, 45, 55, 65 and 75 °C, Ulloa et al (2015) 30 °C, and Aires et al (2023) ranged between 25 °C and 40 °C, with best results at 40 °C. Higher temperatures increased the effectiveness of seed hydration by facilitating ultrasound penetration and increasing the rate of water diffusion. Miano et al. (2018) also observed the combination of temperature (25 ºC - 55 ºC) and ultrasound in the hydration of white beans. They concluded that both improved the process, but the effectiveness of ultrasound decreased with higher temperatures (45 °C and 55 °C), suggesting the combination of both parameters has an antagonistic effect.

The hydration parameters can be modeled to adjust the hydration kinetics data of the DCS, the most used being Peleg, Weibull, Kaptso, and first-order, among others. With this, it is possible to systematize the parameters evaluation involved in hydration kinetics. Ulloa et al. (2015), through mathematical modeling using the Peleg model, we can see a reduction in *K1* by up to 93.0% and an increase in *K2* for the Peleg model. A reduction of *K1* implies a faster process rate, whereas a *K2* refers to a shorter time to reach the maximum water absorption capacity.

Table 1: Effect of ultrasound treatment on seed hydration.

Raw Material	Treatment conditions	Hydration time	Effect on hydration kinetics	Reference
Bath Ultrasound |				
Beans (*Phaseolus vulgaris*)	Frequency: 40 kHz; Power: 130 W/L; T: 30 °C Time: 10, 20 and 30 min.	Hydration after treatment at 30°C until equilibrium humidity.	- Reduction of immersion time (up to 58.8%); - K increase for sigmoid and first-order models - Reduction of *K1* (up to 93.0%) and increase of *K2*, for the Peleg model.	Ulloa et al. (2015)
Sorghum (*Sorghum bicolor*)	Frequency: 40 kHz; Power: 26 W/L; T: 25 °C.	Ultrasound-assisted hydration up to M_∞	- Reduction of hydration time (up to 40%); - Increased initial rate of water intake (12%); - Peleg's model: Reduction of *K1* (42%) and *K2* (78%).	Patero & Augusto (2015)
Mung beans	Frequency of 25 kHz; Power: 41 W/L; T: 25 °C.	Ultrasound-assisted hydration °C up to M_∞ (~10h)	- Reduced hydration time (25%); - Increase *of K* (up to 44%) and reduction of τ (up to 28%) for the Kaptso model.	Miano et al. (2016)
Corn	Frequency: 25 kHz; Power: 41 W/L;	Post-treatment hydration up to M_∞	- Reduction of hydration time (35%, almost 300 min); - Proposed model: increase of (5%), parameter "	Miano et al. (2017)

	T: 25 °C; Time: 300 min (5h).	(~14 h).	$M_{\infty} p$" and "*K8*" did not change significantly and increase of *K9* (30%).	
Pinto beans (*Phaseolus vulgaris)*	Frequency: 25 kHz; Power: 91 W/L; T: 25 °C.	Ultrasound-assisted hydration up to equilibrium moisture.	- Increased equilibrium moisture content (M_{∞})by 3%; - Kaptso's model: increase in *K* (up to 37%) and τ (30%).	Miano & Augusto (2018a)
White kidney beans (*Phaseolus vulgaris)*	Frequency: 40 kHz; Power: 28 W/L; T: 25, 35, 45 and 55 °C.	Ultrasonically assisted hydration up to equilibrium moisture	- Reduction of hydration time (up to 6x); - Kaptso's model: k reduction (up to 22%) and τ *increase* (up to 25%); - The parameters were enhanced with the increase in temperature.	Miano et al. (2018)
Rice (viz. rice paddies)	Frequency: 40 kHz: Power: 50 W/L: T: 25 and 40 °C	Intermittent ultrasound: 10 minutes with an interval of 1 hour up to 12 hours of immersion.	- Increase in the rate of water absorption (up to 60%); - Reduction of the imbibition process (50%); - Decrease of *K1* and *K2* for the Peleg model.	Kalita et al. (2021)
Pantanal Rice *(Oryza latifolia*)	Frequency: Power: T: 35, 45, 55, 65	Hydration of 3 hours, 30 min of treatment and hydration of 10 h,	- Reduction of hydration time (58%, 3h); - Decrease of *K1* (up to 3x) for the Peleg model.	Aires et al. (2023)

	and 75 °C.	60 min of treatment.		
Barley Grains |	Frequency: 25 kHz; Power: 91 W/L; T: 20 °C.	Ultrasound-assisted hydration up to equilibrium humidity (41.2%(w.b.), ~11h).	- Reduction of hydration time (38.01%); - Peleg model: No variation in *K1* and decrease in *K2* (8.6%) and increase *in* M_{eq} (7.6%).	Carvalho et al. (2024)
		Probe Ultrasound |		
Wheat grains (*Triticum* spp.)	Frequency: 40 kHz; Power: 300 to 1500 W. Temperature: 15, 20 and 25 °C	Hydration-assisted up to 0.75 g of water per 100 g of dry matter	- Reduction of hydration time (up to 40%); - *K2* and *Xeq* constant in the Peleg model and decrease of β in the Weibull model. - US in combination with temperature improved the process by up to 72%;	Guimarães et al. (2020)
Chickpeas |	Frequency: 26 kHz; Power: 45.8-108.7 W cm−2; T: 20°C	Hydration - assisted between 10-30 min.	- Reduction of hydration time (90%); - Peleg model: *K1* decreased with increasing power intensity and temperature.	Bender et al. (2024)

*Peleg's model: equilibrium humidity (*Xeq*), mass transfer rate (*K1*) and maximum water absorption rate (*K2*); Weibull model: (*α)* and (*β);* Kaptso's model: hydration rate (*K*), lag phase (τ) and equilibrium moisture content (*MM p* ∞); Proposed model: equilibrium moisture content (∞); The p' p" which means the fraction of water that is absorbed in the fast/short process; *k* 8 is related to the fast/short process, when(*k8) and related to the slow/long process (k9).*

4.3.2. Impact on germination phase II and III

In stage II of germination, seed metabolism is activated. This results in biochemical changes within the seed, including proteolysis and activating digestive enzymes that break down starch stores into sugars and other smaller molecules (Ali & Elozeiri, 2017). The energy released from these reserves drives radicle growth in phase III (Ali & Elozeiri, 2017). Ultrasound can stimulate the metabolic activity of seed cells, speeding up this process (Chen et al., 2020; Foschi et al., 2023). Some studies have revealed the positive impact of ultrasound application on germination percentage, root length, and shoot yield, among others (Table 2).

Increases in germination rate of 18.07% were observed in soybeans (Yang et al., 2015), 35% in wheat grains (Mihaylova et al., 2021), 175.1% in bean grains (Chiu et al., 2021), 49.02% in soybeans (Chen et al., 2023), and 77.09% in bean beans (Yu et al., 2024). The observed results indicate that ultrasound treatment can increase the germination rate of seeds, but the effects vary according to the time of application, ultrasound intensity, and time.

Mihaylova et al. (2021) applied the ultrasound treatment after 48 hours of hydration of wheat seeds, using a power of 50 W for up to 21 minutes. This method resulted in up to a 35% increase in germination rate. The application of ultrasound after hydration may have allowed the water already absorbed by the seeds to facilitate ultrasound penetration, improving germination (Foschi et al., 2023). On the other hand, Yang et al. (2015) applied ultrasound during the hydration of soybean seeds with powers of 100, 200, and 300 W for 30 minutes. There were no significant differences in germination rate between the 100 and 200 W treatments, but the application of 300 W resulted in a significant increase of 18.07% in the germination rate. The higher power probably provided a more effective stimulation of germination due to the acoustic vibration and mechanical energy in the weakening of the seed coat (Ampofo and Ngadi, 2020). Chen et al. (2023) also applied ultrasound during soybean seed hydration, but with a power of 240 W for 60 minutes. This treatment resulted in a 49.02% increase in germination rate, demonstrating that longer exposure to ultrasound can be more effective compared to the previous study.

Studies by Yang et al. (2015) and Chen et al. (2023) have revealed remarkable results regarding bud and root length. Yang et al. (2015) observed that with a power of 300 W applied for 30 minutes, there was a significant increase of 24.4% in bud length, but a detrimental impact after sonication treatment was observed on root length, with a reduction of 20.4%. Chen et al. (2023) recorded a very expressive growth, both of the shoots and the roots, with increases of 237.1% and 267.4%, respectively, using a power of 240 W applied for 60 minutes.

Both studies used soybean seeds as raw material; however, the differences in the ultrasound parameters, mainly the potency and the time of application, influenced the results in different ways. The significant increase in bud length observed by Yang et al. Yang et al. (2015) at 300 W suggests that higher wattage may stimulate early bud growth. This can be explained considering thar high power can also induce stress on the roots, possibly due to excessive ultrasound pressure, influencing the reduction in root length.

On the other hand, Chen et al. (2023) applied a slightly lower power (240 W) for a longer time (60 minutes), resulting in expressive growths of both the bud and the root. This suggests that moderate potency combined with a more prolonged exposure time may be more beneficial for the overall development of the plant, promoting balanced growth without causing excessive stress to the roots.

Table 2: Effect of ultrasound treatment on seed germination.

Raw Material	Treatment conditions	Germination time	Effect on germination	Reference
Bath Ultrasound |				
Soybean seeds	Hydration-assisted (US). Frequency: 40 kHz; Power: 100 to 300 W/L; T: 25 °C; Time: 30 min.	30 °C for 5 days	- Increase in sprout germination rate (↑ 18.07%); - Increase in average bud length (↑24.42%); - Decrease in average root length (↓20.41%).	Yang et al. (2015)
Common bean 'Kabulengeti'	Hydration-assisted (US). Frequency: 40 kHz; Power: 360 and 180 W/L; T: 25°C; Time: 30, 45 and 60 min.	25°C for 24, 48, 72 and 96 h	- Longer radicle length (↑72.37%); - Hypocotyl growth (↑60.44%); - Maximum germination after 36 hours of germination (↑100%); - Increased germination rates and sample vigor.	Ampofo & Ngadi (2020).
Wheat Seeds	Hydration (2 days) and then sonicated. Frequency: 42 kHz; Power: 50 W/L; Time:	22 °C for 9 days	- Increased germination energy (↑46%); - Increased germination rate (↑35%); - Increased seedling growth (↑33%).	Mihaylova et al. (2021)

	3, 9 and 21 min.			
Adzuki beans *(Vignaan gularis)*	Hydration-assisted (US) Frequency: 28 kHz, 40 kHz or 80 kHz; Power: 300 W/L; T: 25 °C; Time: 10 min.	20°C for 6 days	- Increase in germination percentage (↑ up to 175.1%); - Shorter average germination time (↓2.78 days); - Longer hypocotyl lengths, longer radicle and higher bud yield (US-40 kHz).	Chiu et al. (2021)
Soybean seeds	Ultrasound-assisted hydration. Frequency: 60 kHz, 20/60kHz, 20/40/60kHz; Power: 240W/L; Time: 60 min.	25 °C for 96 hours	- Increase in germination percentage (↑49.02%); - Germination percentage increased (↑100%) - Dual frequency (20/60 kHz) provided the longest buds (7.82 cm) and roots (6.32 cm).	Chen et al. (2023)
Red Kidney Bean Kernels	Hydration-assisted (US) Frequency: 20 kHz; Power: 250-450 W/L; Time: 10 min; T: 25°C		- Increased germination rate (↑77.09%); - Increased bud length (↑81.13%)	Yu et al. (2024)
Probe Ultrasound |				

Seeds of *Hymenaea courbarilL.* (jatobá)	Ultrasound-assisted hydration. Frequency: 3 MHz; Intensity of 2 W/cm²; T: 30 °C and 35 °C; Time: 2, 3 and 4 min.	25 °C for 7 days (168 h)	- Increase in %GER (↑10.2%, 3 minutes of sonication); - Decrease in %GER (↓70.08%, 4 min); - Reduction of SMR (↓50.7%, 2 min);	D'ávila et al. (2021)
Tomato, turnip, corn and watermelon seeds	Ultrasound-assisted hydration. Frequency: 19.8 kHz; Power: 200 W; Time: 2.5, 5, 7.5, 10, 12.5 and 15 min.	24 °C for 14 days (tomato, turnip and corn) or 8 days (watermelon).	- Increased germination rate (↑43% tomato and 20% corn); - Reduction of SMR (↓19% tomato and 17% corn); - Increase in seedling emergence (↑41% in corn) - Increase in the vigor index in tomatoes, turnips and corn (up to 121%); - Increase in seedling height and biomass (121% and 188%, respectively) and corn (26% and 23%, respectively).	Nogueira et al. (2023)
Rice cultivars *viz.*	Ultrasound-assisted hydration. Frequency: dual 20-40 kHz; Time: 1.5 min.	28°C in the dark for 7 days	- Increase in germination (↑1.35%) and seed growth (↑4.24%); - Increased germination potential (↑30.00%).	Huang et al. (2024)

*%GER (germination percentage); TMG (average germination time).

4.3.3. Impact on the quality of sprouted flour

Table 3 presents studies that address the effect of ultrasound on the quality of germinated seed meal under different process conditions. Regarding nutritional value, germination can alter the concentration of certain nutrients, such as GABA, proteins, reducing sugars, and antioxidants (Yang et al., 2015; Singh & Sharma, 2017).

γ-aminobutyric acid (GABA) is a stress response factor in plants during germination (Ramesh et al., 2017). Known as a non-protein amino acid, it draws attention because of its numerous physiological functions, such as regulating blood pressure, protecting against cancer, boosting immunity, improving brain function, and delaying the degradation of intelligence (Ding et al., 2018). Several studies have suggested that ultrasound could contribute to an increase in the GABA content of sprouts (Table 3). The increase in GABA content was 43.39% in soybeans (Yang et al., 2015), 31.44% in oats (Ding et al., 2019), 60.40% in beans (Yu et al., 2024), 132% in mung beans (Banura & Singh, 2023), and 182.1% in sorghum (Deore et al., 2023). The increased availability of free amino acids is due to the partial hydrolysis of proteins by hydrolytic enzymes activated during the germination process, potentiated by ultrasound (Singh & Sharma, 2017).

Changes in chemical components, such as protein and reducing sugars, are also observed during germination and can be potentiated with sonication. For example, sonication can lead to significant increases in protein content, as observed in several studies: soybeans (4.33%) (Yang et al., 2015), soybeans (4.54%) (Chen et al., 2023), kidney beans (46.20%) (Yu et al., 2024), and mung beans (21.4%) (Banura & Singh, 2023). This increase may occur because ultrasonic cavitation activates enzymatic activities during germination, and activated phytase and tannase are able to weaken protein-phytate/tannin bonds, releasing more proteins (Ding et al., 2019). In addition, an increase in reduced sugar content may occur, as seen in rice (11.9%) (Ding et al., 2018) and oats (20.9%) (Ding et al., 2019), as well as a decrease, as seen in soybeans (21.66%) (Yang et al., 2015). The increase in glucose content after ultrasound treatment may be related to the increased activity of starch hydrolytic enzymes (Ding et al., 2018), accelerating starch hydrolysis and improving glucose accumulation during germination. On the other hand, the degradation of sugar during germination can be explained by sugar consumption to provides the energy needed for protein synthesis (Yang et al., 2015).

Other important attributes widely found in seeds are bioactive compounds, which the germination process and ultrasound pretreatment can significantly influence. Phenolic compounds, antioxidant activity, and other bioactives are crucial in health promotion and disease prevention (Teodoro, 2019). Increases in phenolics (2.8%) and antioxidant DPPH (72.45%) were observed (Ding et al., 2019); phenolic compounds (1065.3%), flavonoids (559.9%) and antioxidant activity ABTS (25.57%) and DPPH (13.84%) (Ampofo & Ngadi, 2020); antioxidant capacity (19.41%), phenolic compounds (11.53%) (Chen et al., 2023); and phenolic compounds (232%), antioxidant activity (83%) (Banura & Singh, 2023). This occurs because ultrasound acts as a stress factor, stimulating defense mechanisms and increasing the activity of defense enzymes involved in their degradation and synthesis, leading to the accumulation of these compounds (Ampofo and Ngadi, 2020).

From an industrial point of view, improving the techno-functional properties of flour significantly expands their applications in the food sector. Increased solubility, emulsification, water, and oil holding capacity of flours make them more versatile and efficient in various food products (Saran et al., 2024). While ultrasound treatment promoted an increase in water-holding capacity by up to 53.89% in mung bean (Banura & Singh, 2023), a decrease of 50% was observed for sorghum (Deore et al., 2023). The reduction in WHC has been associated with the release of water during starch degradation at germination, reducing the ability of starch to swell and decreasing the hydration qualities of water such as WHC (Deore et al., 2023). On the other hand, WHC may be associated with an increase in the hydrophobic surface of the protein and denaturation of the molecular structure (Zuñiga-Salcedo et al., 2019), consequently increasing the moisture absorption rate.

Structural properties can also be influenced by sonication during germination. Ultrasound treatment and germination can make the surface rougher due to shear forces and localized heating, which intensify the effects of germination (Bai et al., 2023). Changes in particle size can result from the mechanical and thermal effects of ultrasonic waves, strengthening the intermolecular force and leading to the aggregation of starch granules and proteins and an increase in particle size (Bonto et al., 2021). In addition, ultrasound can increase the crystallinity of starch. Liang et al. (2021), linear chain starch can rearrange into crystalline and non-crystalline regions after ultrasound treatment, resulting in a more ordered structure.

Table 3: Effect of ultrasound treatment on sprouted flour quality.

Raw Material	Treatment conditions	Effect on flour quality	Reference
Bath Ultrasound |			
Soybean seeds	Hydration-assisted (USA); Frequency: 40 kHz; Power: 100, 200 and 300 W; Time: 30 min. Germination: 30 °C for 5 days.	- Increase: protein (4.33%), moisture (7.17%), essential amino acids (18.28%) and GABA (43.39%); - Decrease: lipids (27.41%) and sugars (21.66%).	Yang et al. (2015)
Red Rice (GRR) and Brown Rice (GBR)	Hydration-assisted (USA); Frequency: 25 kHz; Power: 16 W/L; T: 23-24°C; Time: 5 min. Germination: 28 °C for 8, 24 and 36 hours.	- Modification of the microstructure; - Increase: starch hydrolysis; sugar reducer (11.9%); ΔE; - Reduction: L* and viscosities determined by an RVA.	Ding et al. (2018)
Oatmeal	Treatment (US) before hydration; Frequency: 25 kHz; Power: 16 W/L; Time: 5 min. Germination: 24 °C for 24, 48, 72 and 96 h.	- Increased GABA (31.44%); - Increase in phenolics (2.8%, 72h) and antioxidant DPPH (72.45%, 96h); - Increased fructose (29.4%), glucose (20.9%), maltose (60.5%) and sucrose (1.1%).	Ding et al. (2019)

Common bean 'Kabulengeti'	Hydration-assisted (USA); Frequency: 40 kHz; Power: 360 and 180 W; T: 25°C; Time: 30, 45 and 60 min. Germination: 25 °C for 24, 48, 72 and 96 h.	- Increase: phenolic compounds (1065.3%), flavonoids (559.9%) and antioxidant activity ABTS (25.57%) and DPPH (13.84%).	Ampofo & Ngadi (2020).
White wheat grains (*Triticum aestivum* L.)	Hydration-assisted (USA); Frequency: 22 kHz; Power: 630 W; T: 20 °C; Time: 8 hours and 16 hours. Germination: 22 ± 2 °C for 120 hours.	- Increase: GABA, antioxidant activity and flavonoids; more uniformly sized particles; - Decrease: grain hardness, damaged starch grains, starch (18.7%);	Naumenko et al. (2022)
Soybean seeds	Hydration-assisted (USA); Frequency: 60 kHz, 20/60kHz, 20/40/60kHz; Power: 240W; Time: 60 min. Germination: 25 °C for 96 hours.	- Increase: antioxidant capacity (19.41%), phenolic compounds (11.53%), proteins (4.54%); protease, amylase and lipase activity. - Reduction: lipids and carbohydrates.	Chen et al. (2023)
Barley	Frequency: 40 kHz; Power: 240 W Temperature: 24 °C; Time: 40 min	- Sparser starch grains after ultrasound; - Aggregation of starch leading to an increase in particle size; - Increase the hydrogen bond in starch and its crystallinity	Bai et al. (2023)
Sorghum	Treatment before 12 h immersion Frequency: 40kHz; Time: 10, 15 and 20 min. Germination: 24 °C for 24 hours.	- Decrease in WAC (50%), with no changes in OAC; - Increase in GABA (182.1%); - Decrease of L* (3.76%), increase of a* (48.49%) and increase of b* (17.86%).	Deore et al. (2023)

Red Beans	Treatment after 12-h immersion; Frequency: 20 kHz; Power: 250 W, 350 W and 450 W; T: 25 °C, Time: 10 min	- Increase in total protein (46.20%) and soluble protein (21.47%); - Decrease in fat content; - Increase in phenolic compounds (42.78%), flavonoids (1.68) and ascorbic acid (1.22); - Increase in GABA (69.40%); - Increase in DPPH (1.08%), ABTS (1.06%) and the chelating capacity of Fe 2+ (1.23); - Increased protein digestibility (64.21%)	Yu et al. (2024)
Probe Ultrasound |			
Brown Rice Grains	Treatment (US) before hydration; Frequency: 28 kHz; Power: 400 W; Time: 5, 10, 15 and 30 min. Germination: 37 °C for 32 hours.	- Increase: reducing sugar, particle size distribution, GABA, antioxidants, proline, free amino acids, and calcium and iron. - Decrease: starch and diameter of starch granules;	Xia et al. (2020)
Mung beans	Ultrasound-assisted hydration. Frequency: 40 kHz; Amplitude: 30%; T: 4 °C; Time: 10, 15 and 20 min. Germination: 24 °C for 18 h.	- Increase: protein (21.4%), GABA (132%) and WAC (53.89%); glucose (223%), sucrose (203%), phenolic compounds (232%), antioxidant activity (83%), viscosity and bonding properties of starch. - Decreased: phytic acid (87%).	Banura & Singh (2023).

5. Future Perspectives and Research Directions

The main goal of applying ultrasound in seeds is to promote improvements throughout the germination process. This technique has been shown to accelerate hydration, significantly increase germination rate and root growth, and improves germinated seeds' nutritional and biological properties (Ding and Feng, 2019). Regardless of the proficiency of this process, to promote the widespread utilization of ultrasound treatment on germinated seeds, it is still necessary to focus on optimized process conditions, seed varieties, and industrial-scale production (Li et al., 2022).

Many studies delve into distinct process variables, such as frequency, potency, exposure time, and treatment steps. However, these studies often provide vague or incomplete descriptions of the processing steps, and the results sometimes need to follow a trend line. This hampers the optimization potential of these processes and, consequently, impacts the properties of the seeds in various ways. Moreover, only some studies consider aspects such as the cost of acquiring the equipment, the operating costs involved in the germination stages, environmental impacts and the feasibility of application on an industrial scale. Therefore, thoroughly and meticulously evaluating these conditions is paramount for effectively advancing ultrasound technology in seed germination and optimizing these processes.

However, in the context of the ongoing quest for nutritional alternatives that can add value to the food industry, the application of ultrasound in seed germination presents exciting opportunities for innovations in the sector (Benincasa et al., 2019). Flours, especially sprouted flour, have a myriad of applications in the development of new nutritious and additive-free products, aligning with the current trends of healthier and clean-label food consumption (Ding and Feng, 2019). These studies are particularly intriguing because, despite the limitations of germination, sprouted flour is gaining popularity as a versatile and nutritious ingredient. It is highly valued in the food industry for its nutritional benefits, enhanced taste, and functional properties (Benincasa et al., 2019).

Figure 7It illustrates the applications of sprouted flour in the food industry. For example, sprouted flour can be consumed directly as traditional sprouted foods in cold dishes, salads, and porridge. In addition, it can be used as a nutritional food ingredient in bakeries and the beverage industry (Cáceres et al., 2019). Both dairy products and non-dairy substitutes can be formulated with sprouted flour to achieve high nutritional value and good sensory acceptability, providing natural and healthy options for vegetarians, vegans, and environmentally conscious consumers (Cáceres et al., 2019).

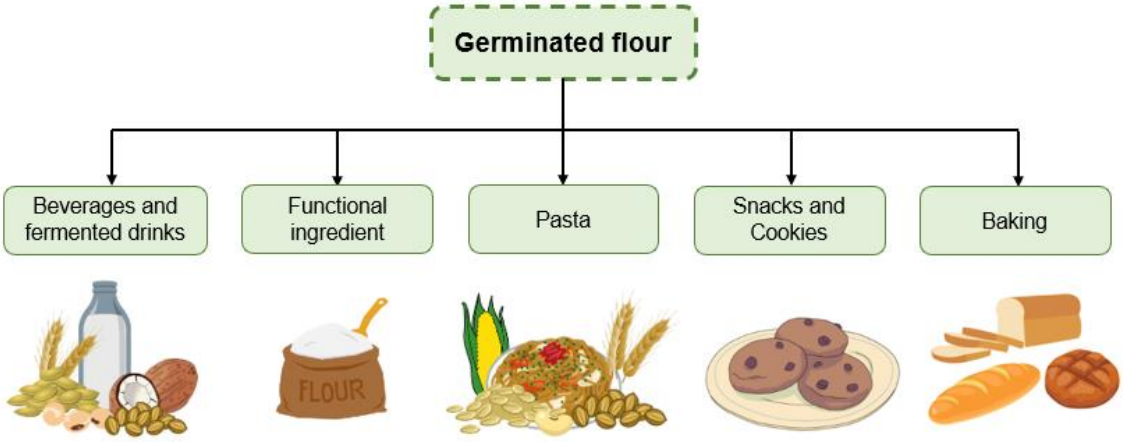

Figure 7. Application of sprouted flours in food products.

In addition to the abovementioned applications, sprouted flour can be used as a functional ingredient to produce foods rich in gamma-aminobutyric acid (GABA). In recent decades, attributed to GABA's important role in human health, researchers have paid great attention to improving GABA contents in various foods, such as seeds (Ding and Feng, 2019).

6. Conclusion

this work used a mixed methodology bibliometric analysis and literature review between 2015 and 2024 to explore the limitations and perspectives of the application of ultrasound technology in seed germination. Bibliometric analysis offers valuable information to agriculture and the food industry on the potential of ultrasound, focusing exclusively on this subject area. The results presented in this work demonstrate several positive effects, such as the optimization of hydration kinetics, improvements in the germination process, and greater availability of nutritional and biological constituents in the sprouted flour.

However, although ultrasound appears in several studies, application methodologies are not yet fully optimized, and process parameters vary significantly among authors, which makes it difficult to define suitable conditions for industrial-scale implementation. Therefore, there is an urgent need for additional scientific research to better understand the impact of process variables on germination, nutritional quality and bioavailability of flour compounds, as well as to optimize processing operating conditions, considering the intrinsic quality of parameters and consumer safety.

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Chapter 2

Ultrasound-assisted intermittent hydration of pumpkin seeds: Improving the water uptake, germination and quality of a clean label ingredient

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Chapter 2: Ultrasound-assisted intermittent hydration of pumpkin seeds: Improving the water uptake, germination and quality of a clean label ingredient

Abstract

This work investigated the ultrasound (US)-assisted intermittent hydration kinetics (1–4 cycles of 10 min, 38 W/L, 25 kHz, 25 or 45 °C) of pumpkin seed and its impact on the germination kinetics, as well as on the nutritional and biological quality of germinated flours. Sonication reduced hydration time (≤ 54.3%), decreased the germination lag phase (≤ 19.3%), and increased maximum germination capacity (≤ 5%) ($p < 0.05$). Furthermore, ultrasonic treatment led to greater rootlet length (≤ 19.4%) after germination. Regarding the flour quality, ultrasound increased protein and lipid content (≤ 23% and ≤ 8.4%, respectively), with the best result observed at 45 °C for 4 cycles of 10 min. Additionally, samples sonicated for 2 cycles of 10 min showed higher levels of reducing sugars (≤ 79%), phenolic compounds (≤ 81%), and soluble protein (≤ 19.8%) compared to non-US-treated germinated flour. These findings highlight the potential of ultrasound-assisted hydration, especially above two intermittent sonication cycles, for optimizing pumpkin seed germination and enhancing flour quality.

Keywords: Emerging technologies · Germination · Hydration · Nutritional quality · Pumpkin seeds · Sonication

1. Introduction

A growing strategy is to valorize agro-industrial by-products, such as peels and seeds, as functional ingredients for food applications (Noh et al., 2022). However, processes need to be developed to transform these biomasses, enhancing their properties, and thereby obtaining quality ingredients with a clean label.

Pumpkin (*Cucurbita moschata* D.) is widely cultivated in Mexico, China, South Asia, Africa, India, Latin America, and the United States, with an annual production of 27 million tons (Grassino et al., 2023). This vegetable is consumed in meals or used industrially to produce soups, purees, sweets, and jams, among others (Pacheco et al., 2024). Although pumpkin seeds are often discarded during processing (Pacheco et al., 2024), they contain high levels of lipids (37.8%-45.4) and proteins (25.2%-37.0%), as well as bioactive compounds such as phenolic compounds, which encourage their exploration in the food industry (Singh & Kumar, 2024).

Bioprocessing techniques, such as germination, can improve the seed nutritional and functional qualities, reduce anti-nutritional factors, and increase the digestibility of macro and micronutrients, especially proteins and lipids (Lemmens et al., 2019). Before germination, seeds must be hydrated (Liu et al., 2023). Hydration is a complex phenomenon that involves diffusion and capillarity of water, resulting in different absorption behaviors (Louf et al., 2018), being also long (up to 48 hours) and expensive (Kalita et al., 2021). Therefore, there is great interest in accelerateng this process from an industrial point of view.

Different unconventional technologies, such as ultrasound (US), have been studied to reduce the hydration time of grains and seeds (Miano & Augusto, 2018). For example, studies have shown that ultrasound can increase the water absorption rate of pinto beans (*Phaseolus vulgaris*) (Miano et al., 2018), Andean lupine grains (Miano et al., 2019), wheat grains (Guimarães et al., 2020), durum wheat (Yildirim et al., 2022) and rice (Aires et al., 2023). It can also have positive impact on the germination of mung bean grains (Miano et al., 2016), Adzuki beans (Chiu, 2021); *Pinus tabuliformis* seeds (Zhang et al., 2023) and rice seeds (Huang et al., 2024). However, studies are lacking regarding the application of ultrasound-assisted hydration in grains/seeds, its impact on germination kinetics and, in special, the quality of the resulting flour. Flours obtained from germinated grains can be used as clean-label ingredients for different applications, such as bakery, roasts, tortillas, biscuits, cookies, muffins, snacks, cereal bars, and water-soluble extracts, this being a subject of growing demand (Finnie et al., 2019). Thus, this study presents a novel approach, aiming to evaluate the hydration kinetics of pumpkin seeds assisted by ultrasound (38 W/L and 25 kHz), applied intermittently at different times (up to 4 cycles of 10 minutes) and temperatures (25 and 45 °C), as a strategy to obtain flours for food application. In addition, this study also investigates the impact on the seed germination properties, and the nutritional and biological properties of the obtained flours, with the goal of increasing their applicability in the food industry.

2. Materials and methods

2.1. Raw material and general description of the process

Pumpkin seeds (*Cucurbita moschata* D.) were supplied by the company Doces Mirahy (Miraí, Minas Gerais, Brazil). After obtaining the seeds, residues and impurities were removed, followed by drying at 45 °C for 24 hours. Subsequently, the seeds were vacuum packed and stored at a temperature of 7 °C until the beginning of the study.

The seeds were then hydrated, with and without the assistance of ultrasound technology, germinated, dried and ground, thus obtaining flours with the potential use of clean label ingredients. The samples were characterized in relation to their nutritional and bioactive content, as described below.

2.2. US-assisted pumpkin seed hydration process

For each treatment, approximately 40 g of pumpkin seeds were immersed in a glass beaker containing a 200 mg L^{-1} sodium hypochlorite solution for 15 minutes to sanitize them. Then, the sanitized pumpkin seeds were drained through sieves and washed under running water to remove residues from the sanitization process. Subsequently, the seeds were placed in a bottle with a ratio of 1:3 (seed: water) to continue the hydration process (Fig. 1).

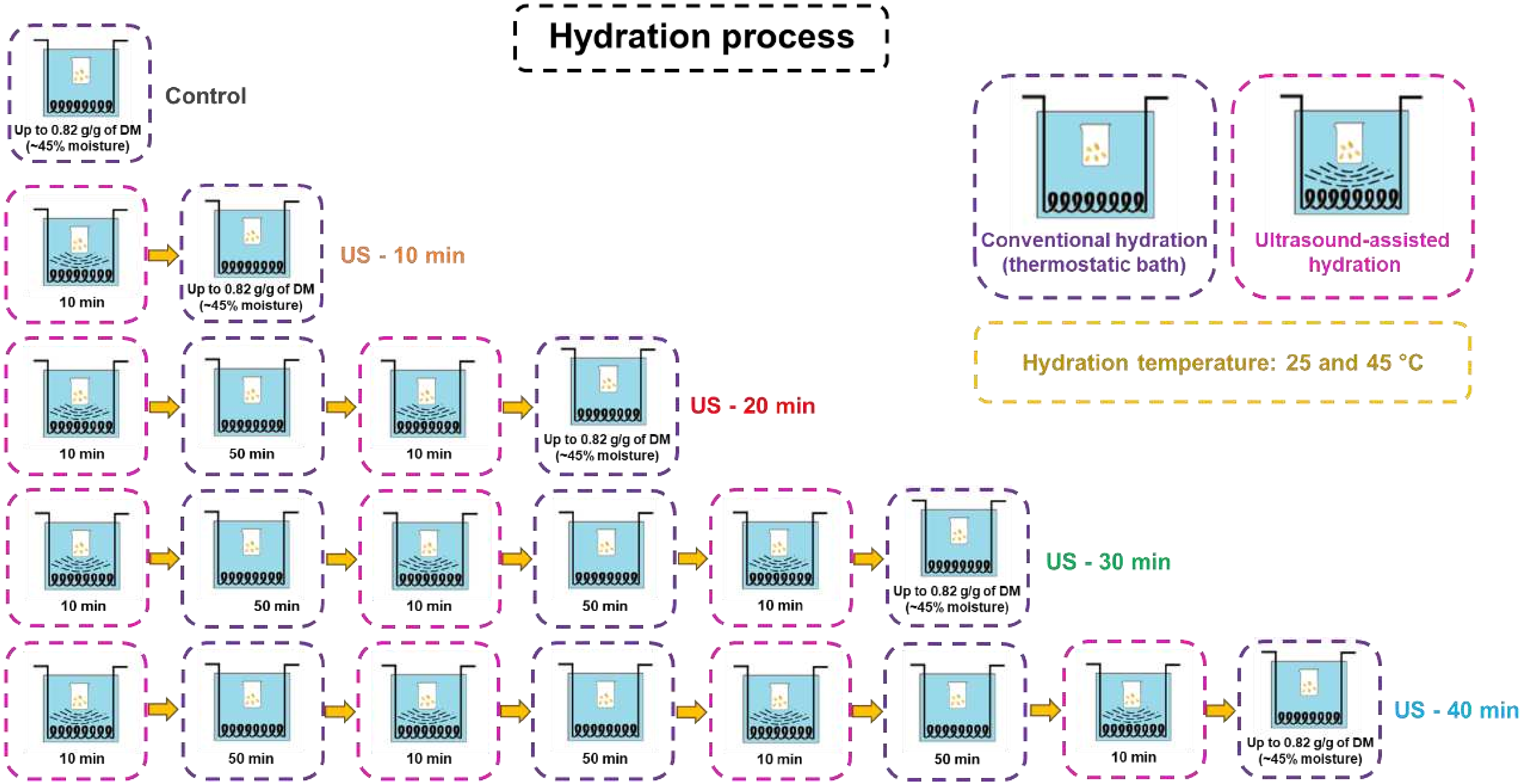

Fig. 1 Flow chart of the work

Conventional hydration was carried out in a thermostatic bath (SSDu 10L, Solidsteel, Piracicaba, Brazil). An ultrasonic bath (Unique, model USC 2800 A, Brazil) with a nominal power of 450 W and volumetric power of 38 W/L was used for the US-assisted hydration process. The bath was operated at a frequency of 25 kHz, with temperature control using a stainless-steel heat exchanger inserted inside the ultrasonic bath with water recirculation provided by an external thermostatic bath. In both cases, the hydration was carried out at 25 ± 2 °C (optimum temperature for pumpkin seeds germination, according to Zehtab-Salmasi (2006) and 45 ± 2 °C (highest temperature for pumpkin seed germination, according to Ghaderi et al. (2008)).

Sonication was performed during hydration intermittently, at different cycle times: US – 10min (1 cycle of 10 minutes on US), US – 20min (2 cycles of 10 minutes on US interspersed with 50 minutes in a thermostatic bath), US – 30min (3 cycles of 10 minutes US interspersed with 50 minutes in a thermostatic bath) and US – 40min (4 cycles of 10 minutes US interspersed with 50 minutes in a thermostatic bath), as illustrated in Fig. 1. The first sonication cycle was performed at the beginning of hydration. Instead of the continuous application of ultrasound, this approach was chosen to limit the associated costs and complexity of industrially using this technology (such as energy consumption for ultrasound and cooling, rational use of equipment, limiting wearing, etc.). We highlight this approach can better represent a possible industrial application. Therefore, we chose to evaluate the relatively short sonication time (10 min, 16.7% of each hour) and the impact of the process applied at different points of hydration, which explains the sonication intervals rather than applying continuous US at the beginning of process.

Regardless of the sonication cycles applied, the seeds were kept under hydration conditions in thermostatic bath until reaching the moisture content of 45% (0.82 g/g of DM), established as the moisture required to break seeds dormancy (Bryce et al., 2010).

2.3. Hydration kinetics

The hydration kinetics were conducted according to the methodology described by Miano and Augusto (2018), which involves determining the moisture content of the seeds through a mass balance using the initial moisture content. The increase in moisture was monitored every 15 minutes for 1 hour and, subsequently, every 60 minutes for the remaining time intervals. After hydration, the water was removed using a sieve, and the excess moisture in the pumpkin seeds was absorbed with paper towels before proceeding to the germination process.

To evaluate the hydration kinetics, the Peleg model (Equation 1 (1)) and the Weibull Model (Equation 2 (2)) were used.

$$X(t) = X_0 + \frac{t}{K_1 + K_2 t} \qquad (1)$$

Where *X(t)* is the moisture content (g of water per g of dry matter) at time t, X_0 is the initial moisture content (g of water per g of dry matter), t is the hydration time (h), and K_1 (g of dry matter. h g^{-1} · of water) and K_2 (g of dry matter. g^{-1} of water) are model parameters.

$$\frac{X(t) - X_0}{X_{eq} - X_0} = 1 - exp\left[-\left(\frac{t}{\beta}\right)^{\propto}\right] \quad (2)$$

Where *α* is the shape parameter (dimensionless), which is also associated with the initial rate of water absorption, and *β* is the time scale parameter (h), which represents the kinetics (and the time needed to reach 63% of o equilibrium moisture content – Guimarães et al., 2020).

Furthermore, according to the constant K_2 of the Peleg model, the equilibrium moisture content (X_{eq}) can be estimated, considering long periods of hydration (t → ∞) (Equation 3 (3)) (Guimarães et al., 2020).

$$X_{eq} = X_0 + \frac{1}{K_2} \quad (3)$$

The Peleg and Weibull Model parameters were obtained using the CurveExpert Professional software (v.1.5), with a confidence level of 95%. Model accuracy was assessed by calculating the adjusted coefficient of determination (R^2_{adj}) (Equation 4 (4)) and root-mean-square error (RMSE) (Equation 5 (5)) (Polachini et al., 2023).

$$R^2_{adj} = 1 - \frac{n-1}{n-(k+1)}(1 - R^2) \quad (4)$$

$$RMSE = \sqrt{\frac{\sum_{n=1}^{n}(y_p - y_{exp})^2}{n}} \quad (5)$$

Where *n* is the number of observations, *k* is the number of parameters in the model, R^2_{adj} is the fitted coefficient of determination, y_p is the predicted value, and y_{exp} is the experimental value.

2.4. Evaluation of the germination process of pumpkin seeds previously subjected to the US-assisted hydration process

2.4.1. Germination percentage (%GER), average germination time (AGT), and germination kinetics

After seed hydration reached approximately 45% moisture (0.82 g of water/g of DM) (Bryce et al., 2010), the samples were subjected to the germination process. For this, 40 grams of seeds (~240-250 seeds) from each treatment were arranged without overlapping in polyethylene trays (0.104 m^2) wrapped in three layers of moistened filter paper. The trays were incubated in a BOD (Novaética, 411 - 255 DE, Rio de Janeiro, Brazil) at 25 °C for 72 h in the dark. To control humidity, seeds were sprayed with distilled water three times a day, and relative humidity was calculated based on dry bulb and wet bulb temperatures, remaining in the range of 80-85%.

During the germination process, the germination percentage (%GER) was determined at 0, 6, 12, 24, 36, 48, 60 and 72 hours. This percentage corresponds to the number of germinated seeds divided by the total number of seeds, as given by Equation 6 (6):

$$(\%)\ GER = \frac{n}{N}\ x\ 100 \quad (6)$$

Where *n* is the number of germinated pumpkin seeds, and *N* is the total number. To determine whether a seed germinated, the development of the radicle was monitored. Seeds were considered germinated when the opening of the tegument with root emergence was visible (Lemmens et al., 2019), more specifically, at least 1 mm. Germination was completed after 72 hours of incubation.

Furthermore, the average germination time (AGT) was calculated considering the incubation time of three days, as defined by Equation 7 (7):

$$AGT = \frac{\sum n_i t_i}{\sum n_i} \quad (7)$$

Where n_i is the number of seeds germinated per day, t_i (day) is the incubation time, ranging from 1 to 3 days.

At the same time, to evaluate germination kinetics, that is, the percentage of seeds germinated over time (% GER (t)), the Gompertz model modified by Zwietering et al. (1990) was used (Equation 8 (8)). From this model, the following parameters were obtained: λ (h), which represents the germination lag phase (time needed to start germination); k_g (h^{-1}), which means the germination rate; and G_{max} (%), which indicates the maximum percentage of germination.

$$\%GER\ (t) = G_{max}\ .exp\ \left\{-\ exp\ \left[\frac{\kappa_g\ .\ e}{G_{max}}(\lambda - t) + 1\right]\right\} \quad (8)$$

The parameters of Equation 8 were obtained through non-linear regressions using the CurveExpert Professional software (v.1.5) (95% confidence level), and the model fit was evaluated by the coefficient of determination (R^2).

2.4.2. Radicle development during the seed germination process

To evaluate radicle development during the seed germination process, ten pumpkin seeds were randomly selected from each treatment, and the radicle length (rootlet length) was measured with a professional optical caliper (Mitutoyo, Japan) after 0, 12, 24, 36, 48, 60, and 72 hours of germination. Furthermore, the samples were photographed at the same times to illustrate root development.

2.5. Composition of pumpkin seed flours

2.5.1. Sample preparation

After germination, the pumpkin seeds were weighed and then dried in an oven (Q819V2, QUIMIS, Diadema, Brazil) at 50 °C for 12 hours until they reached a constant weight. The same procedure was performed for the non-germinated sample. After drying, for the germinated seeds, the rootlets were removed, and the germinated and non-germinated seeds were ground in an analytical mill (180 W, model IKA® A11 B S32, Germany). Subsequently, the samples were packaged in vacuum-sealed plastic bags and stored at 7 °C for further analysis.

2.5.2. Proximate composition

The proximate composition of the flours was carried out according to the methods described by AOAC (2005). Moisture content was determined using an oven at 105 ºC until constant mass was reached. The crude protein content was determined using the Kjeldahl method, multiplying the value obtained by the factor 6.25. The ash content was analyzed using a muffle furnace at 600 ºC until constant weight. The lipid content was determined using the Soxhlet method, which used petroleum ether as a solvent. The total carbohydrate content (TC%) was estimated using the difference method, according to Equation 9 (9).

$$\mathrm{TC\%} = 100 - (\mathrm{TM}(\%) + \mathrm{TP}(\%) + \mathrm{TL}(\%) + \mathrm{TA}(\%)) \quad (9)$$

Where TM (%) represents moisture content, TP (%) represents crude protein content, TL (%) represents lipid content, and TA (%) represents ash content.

2.5.3. Reducing sugars

Reducing sugars was quantified by the dinitrosalicylic acid (DNS) method described by Saqib and Whitney (2011). The reducing sugar content was obtained from the glucose standard curve (GE), and the results were expressed in mg of glucose equivalent per gram of sample on a dry basis (mg of glucose/g DM).

2.5.4. Soluble protein

For the quantification of soluble proteins, 3 g of flour from each sample was diluted in 18 mL of water to obtain the plant extract. Then, the extract was processed in a high shear disperser (Ultra-Turrax® disperser, model T-18 Basic, Ika, Germany) at 12,500 rpm for 30 seconds at 23 °C. Subsequently, 2 mL of plant extract was centrifuged at 10.000 rpm for 20 min, and 1 mL of the collected supernatant was diluted 100 times. The absorbance reading was performed at a wavelength of 750 nm using a UV-visible spectrophotometer. The soluble protein content was determined by the method of Lowry et al. (1951), and the concentration of soluble protein was expressed in g of protein per 100 g of dry matter (g/100g DM) based on the standard curve obtained with bovine serum albumin.

2.5.5. Total phenolic compounds

The Folin-Ciocalteu (FC) method was used to measure the total phenolic content of pumpkin seed flour according to the method described by Saavedra et al. (2015). The results were expressed in terms of gallic acid content (mg) per 100 grams of pumpkin seed flour dryly (mg GAE/ 100g of DM).

2.6. Experimental design and statistical analysis

The processes were performed in three replicates, and each analysis was performed in triplicate. The results were expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA, followed by Tukey's post-hoc test, to compare the effects between different treatments, with a 95% confidence level using Statistica software, version 9.2 (StatiSoft INC, USA).

3. Results and Discussion

3.1. Ultrasound-assisted hydration of pumpkin seeds

Seed hydration curves at 25 and 45 °C, assisted by ultrasound or not are shown in Fig. 2. Regarding water temperature, it was observed that the increase from 25 °C to 45 °C reduced the hydration time by 13.5% for non-sonicated samples and by up to 17.3 % for sonicates. Higher temperatures increase the kinetics of water diffusion in the seed, expanding and softening the plant's microstructures and facilitating water migration due to breaking molecular bonds (Balbinoti et al., 2022). However, high temperatures can result in desirable, acceptable, or undesirable changes, depending on the purpose of the process. For example, protein denaturation, starch gelatinization, and molecular degradation can occur during high-temperature processing. Furthermore, additional equipment and a greater amount of energy are required to increase the temperature of the water and grain during hydration, resulting in high costs for the industry (Miano et al., 2016).

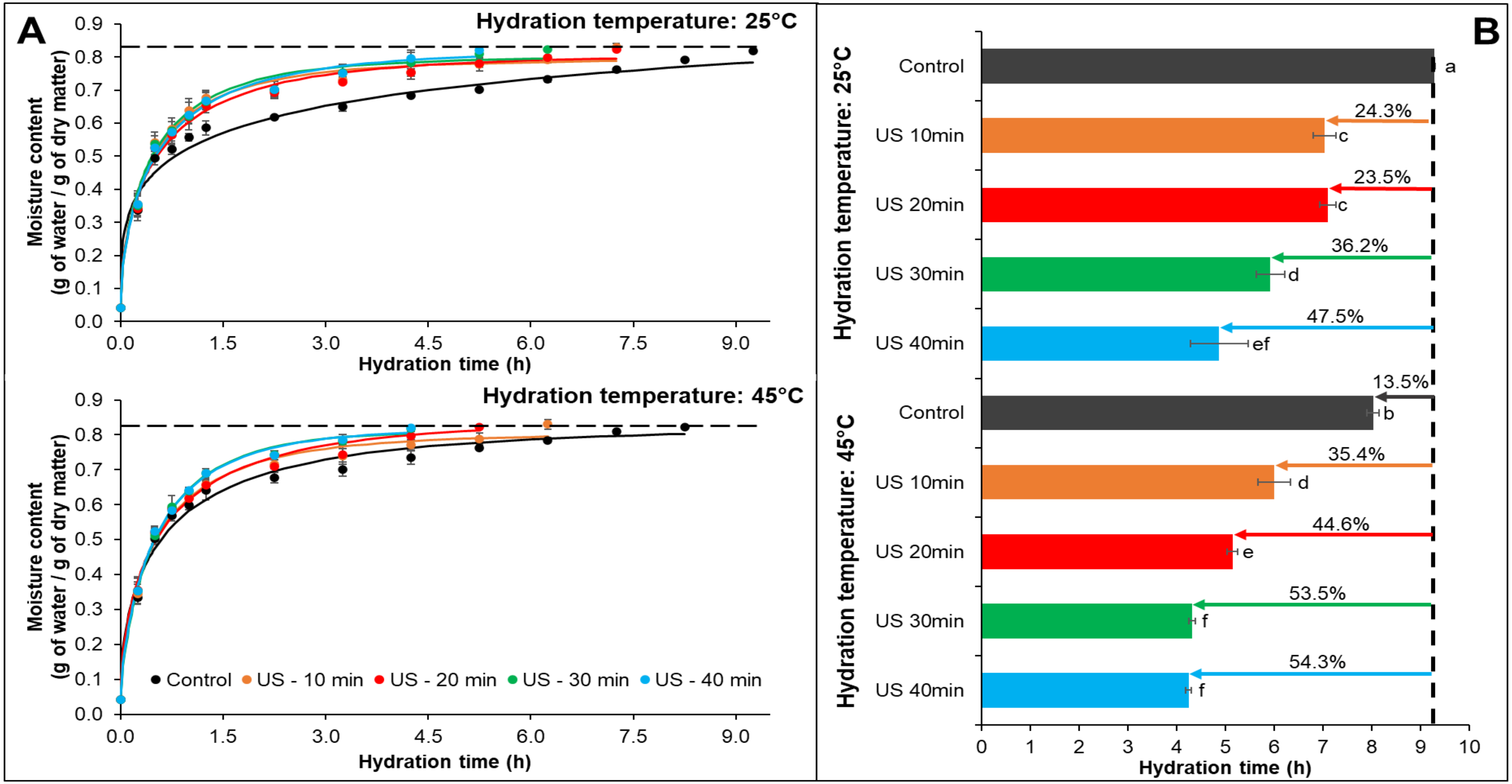

Fig. 2 Hydration kinetics of pumpkin seed assisted by ultrasound and (B) hydration time required by each treatment to reach the moisture content needed for pumpkin seed germination. Different lowercase represents significant difference (p<0.05) by the Tukey test among the treatments. The dots are the experimental values; the bars the standard deviation; and the lines represent the fitted data by Weibull Model (Eq. 2). The horizontal dashed line in (A) represents the moisture of 0.82 g of water per g of dry matter

Regarding the impact of sonication, it was observed that the greater the number of US exposure cycles, the shorter the seed hydration time. Specifically, for samples sonicated for 1, 2, 3, and 4 cycles of 10 min at 25 °C, the time required for hydration was reduced by 24.3%, 23.5%, 36.2%, and 47.5%, respectively, when compared to the control (Fig. 2B). The effect obtained from ultrasound technology in accelerating the hydration process of pumpkin seeds stands out as relevant, considering the approach applied in this work. Even when applying ultrasound intermittently, in cycles, the reductions observed are compatible with the results obtained by previous studies using this technology continuously, throughout the entire hydration process. For example, when US was used continuously, a decrease in hydration time of 40% was observed in sorghum (Patero & Augusto, 2015), 25% in mung beans (Miano et al., 2016), 35% in corn (Miano et al., 2017), 40% in lupine (Miano et al., 2019), 42% in wheat (Guimarães et al., 2020), 40% to 58% in rice grains (Li et al., 2019; Aires et al., 2023). It is worth noting that the effect of US may vary according to the plant studied due to a series of factors related to the structure of the seed, its dormancy mechanisms, and chemical composition, in addition to the ideal conditions of the process (Miano et al., 2018).

Furthermore, process parameters largely influence the effect of ultrasound, such as the way the waves are applied, resulting in acoustic field, frequency, and volumetric power, among others. In general, the approach chosen here, of intermittent application of ultrasound, presents relevant results in intensifying the hydration process while at the same time promoting the rational use of the technology. This is a major advance compared to studies that performed continuous US-assisted hydration. The ultrasonic impact is correlated to the effects of cavitation, sponge effect, and the generation of microjets, which can promote changes in the microstructure of the seed coat and, consequently, enhance the hydration of the vegetable (Kumar et al., 2023). Specifically, ultrasound-induced cavitation generates mechanical pressure on the seeds (Silva et al., 2014) inducing the formation of micropores and microcracks in the cell wall, with a consequent increasing the permeability of the seeds to water and oxygen that facilitates their hydration (Kumar et al., 2023).

In the combined process, that is, increasing the temperature (45 °C) and using ultrasound technology, the reduction in hydration time was up to 13.5% for the control sample and 35.4%, 44.6%, 53.5% and 54.3 % for sonicated samples (1 to 4 US cycles of 10 min, respectively) (Fig. 2B). These results demonstrate that US and temperature, and the combination of both, can enhance ($p<0.05$) the hydration kinetics of pumpkin seeds. However, the effect of ultrasound is smaller at higher temperatures – in fact, when the soaking water temperature increases, the ultrasonic impact can be reduced due to the cushioning effect of the vapor inside the cavitation bubbles (Raso et al., 1999).

Mathematical modeling of hydration behavior is important for better understanding the effects of ultrasound. Table 1 shows the parameters of the Peleg Model (Eq. (1)) and the Weibull Model (Eq. (2)). The R^2_{adj} results were > 0.979 and > 0.986, for the Peleg and Weibull models respectively, and for the RSME, the observed values were < 0.0352 and < 0.0328, respectively, showing that both models presented an excellent fit to the data obtained.

Peleg constant K_1 is related to the inverse of the initial sorption or mass transfer rate (Jideani & Mpotokwana, 2009) when $t \to 0$. The results revealed that K_1 values were not influenced by US application times nor process temperature ($p>0.05$). On the other hand, K_2, which is inversely proportional to the maximum water absorption capacity, decreased ($p<0.05$) with increasing US exposure time for both temperatures, when compared to the control hydration process (maximum reduction of 8.1% when applying 3 and 4 cycles of 10 min of sonication at 25 °C and 11.9% for the same samples at 45 °C). This indicates an increase in water absorption capacity with the application of the US, which is enhanced with increasing temperature. For equilibrium moisture (X_{eq}), the increase in process temperature, as well as the increase in the number of sonication cycles, resulted in higher X_{eq} values ($p<0.05$) (ranging from 5.1% to 12.8%, with greater differences for samples with 3 and 4 cycles of 10 min of US at 45 °C).

Table 1 Fitting parameters of Peleg (Eq. (1)) and Weibull (Eq. (2)) models to the hydration data of pumpkin seeds assisted by US

Peleg Model						
Sample	**Hydration temperature**	***k₁*** **(g of dry matter h g^{-1}· of water)**	***k₂*** **(g of dry matter g^{-1} of water)**	***X_eq*** **(g of water g^{-1} of dry matter)**	R^2_{adj}	***RMSE***
Control	25 °C	0.55 ± 0.05[a]	1.35 ± 0.00[a]	0.78 ± 0.00[c]	>0.979	<0.0352
US 10min		0.43 ± 0.06[a]	1.27 ± 0.01[b]	0.83 ± 0.01[bc]	>0.992	<0.0211
US 20min		0.48 ± 0.06[a]	1.27 ± 0.01[bc]	0.83 ± 0.01[abc]	>0.993	<0.0200
US 30min		0.47 ± 0.10[a]	1.24 ± 0.02[bc]	0.85 ± 0.02[ab]	>0.993	<0.0188
US 40min		0.47 ± 0.07[a]	1.24 ± 0.04[bc]	0.85 ± 0.03[ab]	>0.992	<0.0185
Control	45 °C	0.50 ± 0.03[a]	1.28 ± 0.02[ab]	0.82 ± 0.01[bc]	>0.990	<0.0219
US 10min		0.47 ± 0.05[a]	1.25 ± 0.04[bc]	0.84 ± 0.03[ab]	>0.996	<0.0158
US 20min		0.47 ± 0.05[a]	1.24 ± 0.05[bc]	0.85 ± 0.03[ab]	>0.990	<0.0208
US 30min		0.48 ± 0.01[a]	1.19 ± 0.02[c]	0.88 ± 0.01[a]	>0.991	<0.0197
US 40min		0.48 ± 0.02[a]	1.19 ± 0.02[c]	0.88 ± 0.02[a]	>0.995	<0.0152

Weibull Model					
Sample	**Hydration temperature**	**α (dimensionless)**	**β (h)**	***R^2_{adj}***	***RMSE***
Control		0.33 ± 0.02^b	3.29 ± 1.63^a	>0.986	<0.0286
US 10min		0.63 ± 0.01^a	0.52 ± 0.07^b	>0.989	<0.0256
US 20min	25 °C	0.59 ± 0.07^a	0.62 ± 0.06^b	>0.990	<0.0243
US 30min		0.65 ± 0.09^a	0.52 ± 0.02^b	>0.992	<0.0328
US 40min		0.62 ± 0.11^a	0.61 ± 0.07^b	>0.992	<0.0193
Control		0.54 ± 0.09^a	0.74 ± 0.17^b	>0.989	<0.0260
US 10min		0.62 ± 0.06^a	0.58 ± 0.17^b	>0.993	<0.0201
US 20min	45 °C	0.60 ± 0.18^a	0.67 ± 0.10^b	>0.992	<0.0183
US 30min		0.75 ± 0.13^a	0.57 ± 0.04^b	>0.992	<0.0193
US 40min		0.73 ± 0.10^a	0.58 ± 0.03^b	>0.994	<0.0163

Means followed by different lowercase letters in the same column represent significant difference by the Tukey test ($p<0.05$).

Table 1 also shows the values of the *α* and *β* parameters of the Weibull model. The parameter *α* is related to the mechanisms of water migration from the surroundings towards the center of the seed. According to Corzo et al. (2010), *α* values below 1 indicate that the external resistance to water transfer is much lower than the internal resistance for hydration processes. All *α* values were below 1, confirming that the hydration process of pumpkin seeds is limited by internal water transport rather than external resistance. In the comparative evaluation among the samples, it was observed that all samples presented higher *α* values (≤127%) compared to the control treatment carried out at 25 °C ($p<0.05$), without, however, showing differences between the sonicated samples and control sample at 45 ºC ($p>0.05$). On the other hand, although the *α* values of the sonicated samples were higher, the kinetic parameter *β*, which represents the time (h) necessary to reach 63% of total seed hydration (Guimarães et al., 2020), decreased when the samples were exposed to ultrasound compared to the control sample at 25 °C (reduction of up to 6.3x) ($p<0.05$). This indicates that samples sonicated and hydrated at a higher temperature showed a higher hydration rate ($p>0.05$), reaching final moisture content more quickly than the non-sonicated sample hydrated at 25 ºC (Fig. 2).

3.2. Effect of US-assisted hydration on pumpkin seed germination: Impact on % germination and radicle development

Fig. 3 shows the impact of ultrasound-assisted hydration at 25 °C (Fig. 3A) and 45 °C (Fig. 3B) on the germination of pumpkin seeds, whose kinetics was characterized by typical sigmoidal curves. Fig. 3 also presents the parameters of the Gompertz model (Eq. 9), which adequately adjusted the germination data ($R^2>0.999$). This model provides three parameters related to the germination process: λ, which represents the duration of the lag phase (h) (Fig. 3C), k_g which describes the germination rate (/h) (Fig. 3D) and G_{max}, which refers to maximum germination capacity (%) (Fig. 3E).

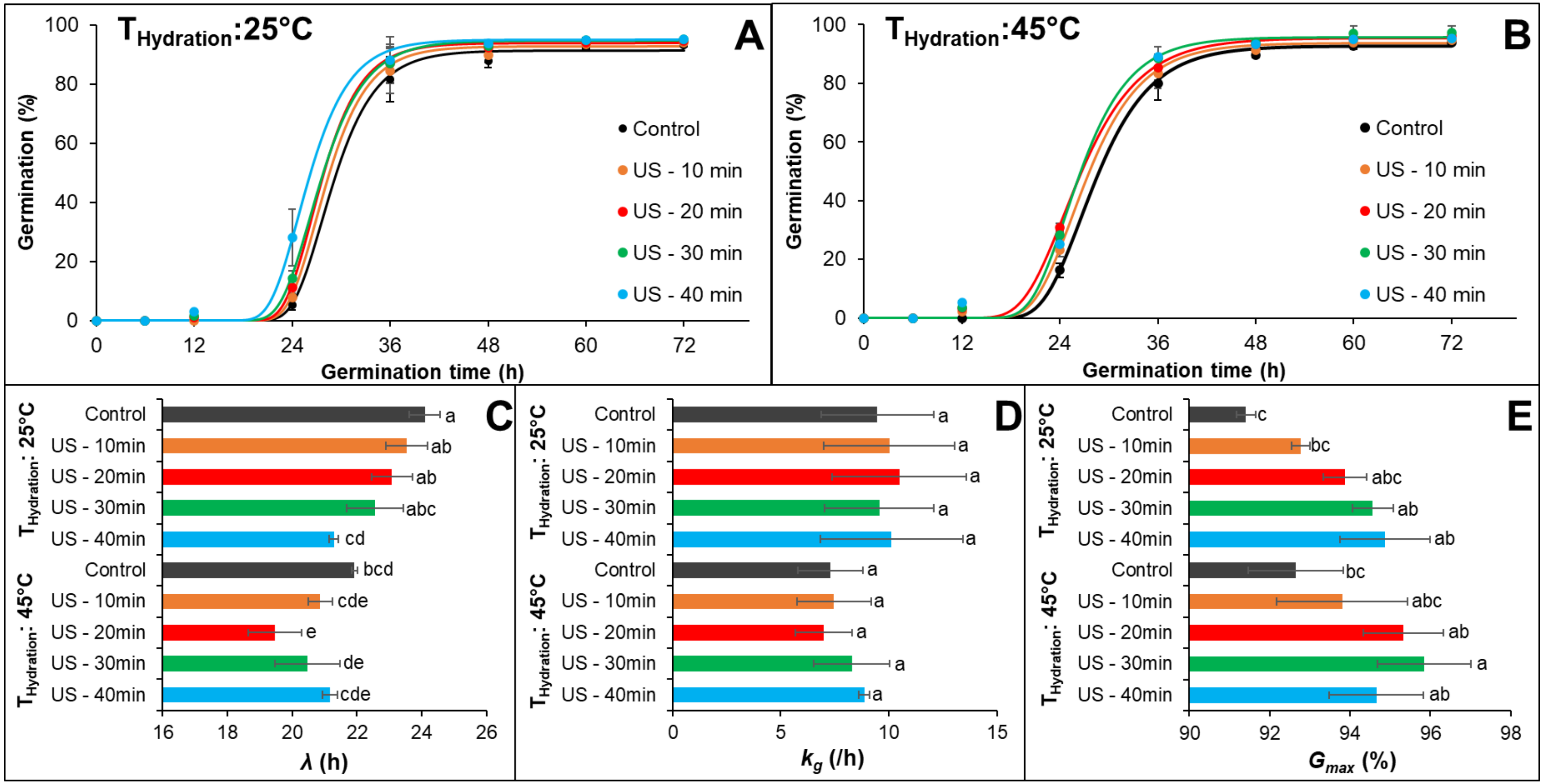

Fig. 3 Impact of ultrasound assisted hydration at 25 °C (A) and 45 °C (B) on the germination kinetics of pumpkin seed at 25 °C and on the parameters of Gompertz model Eq. (9) (C-E). Different lowercase represents significant difference ($p<0.05$) by the Tukey test among the treatments. The dots are the experimental values; the bars represent the standard deviation; and the lines represent the fitted data by Eq. (9). *λ* (h): is the lag phase of germination (the required time to start the germination); k_g (/h) is the germination rate; G_{max} (%) is the maximum germination capacity. R^2: >0.9990

In general, sonication and increasing the hydration temperature enhanced germination, with positive results for the parameters λ and G_{max}. Specifically, the US 40 min / 25 °C and US 20 min / 45 °C samples showed a reduction in the lag phase (decrease of 11.7% and 11.2%, respectively) during germination, compared to their respective control processes ($p<0.05$). This result is probably due to the possible increase in the permeability of the seed coat after the ultrasonic process (Kumar et al., 2023). Before radicle emergence, seeds go through a lag phase, in which enzymes begin their activity using nutrients as sources of energy reserves (Bewley et al., 2012). Through mechanical effects, the US facilitates the passage of water through the membrane, inducing the release of enzymes, which contributes to increased starch degradation (Yaldagard et al., 2008).

Although US-assisted hydration did not affect the germination rate (parameter k_g, Fig. 3D) ($p>0.05$), meaning the samples showed similar slopes at the inflection point, it was observed that samples sonicated for 3 and 4 cycles of 10 min at 25 °C and sample sonicated for 3 cycles of 10 min at 45 °C showed an increase (up to 4.8%) in the maximum germination capacity (G_{max} parameter) ($p<0.05$). These results are corroborated by the average germination time (AGT) and the percentage of germination (%GER) on the first, second, and third days of germination (Table 2).
Based on these results, it was observed that samples hydrated under US, especially with 4 cycles of 10 min at 25 °C and 45 °C, showed a shorter AGT (reduction of up to 12.1% - Table 2). This result is justified by the decrease in the latency phase, which increased the percentage of germination, especially on the first day (an increase of up to 493.3% compared to the control at 25 °C). At the end of the process, however, there was a germination of ~97%, with low variations between the samples sonicated at both temperatures. Considering the lengthy germination times associated with high costs and risks, accelerating this process is interesting from an industrial standpoint.

Radicle development was monitored over the three days of germination. Fig. 4 shows the impact of ultrasound-assisted hydration on the rootlet length (Fig. 4A,B) and visual appearance (Fig. 4C,D) of pumpkin seeds during germination.

Table 2 Impact of ultrasound assisted hydration at 25°C and 45°C on the average germination time (AGT) of pumpkin seed and on the germination percentage (Germination - %) at the first, second and third day of germination at 25 °C

Sample	**Hydration temperature**	**AGT (days)**	**Germination (%)**		
			1st day	**2nd day**	**3rd day**
Control		1.57 ± 0.05^{a}	5.22 ± 1.70^{e}	87.66 ± 2.10^{d}	93.02 ± 0.53^{c}
US - 10min		1.55 ± 0.06^{a}	7.89 ± 2.22^{de}	89.84 ± 2.59^{bcd}	93.97 ± 0.73^{bc}
US - 20min	25 °C	1.52 ± 0.06^{abc}	11.13 ± 1.91^{de}	92.36 ± 0.31^{abc}	94.51 ± 0.37^{bc}
US - 30min		1.48 ± 0.02^{abc}	14.15 ± 2.76^{cde}	92.92 ± 0.74^{abc}	95.21 ± 0.59^{abc}
US - 40min		1.38 ± 0.09^{c}	28.02 ± 9.58^{a}	93.72 ± 1.23^{ab}	95.22 ± 0.57^{abc}
Control		1.51 ± 0.04^{ab}	16.25 ± 2.39^{bcd}	89.72 ± 0.85^{cd}	93.78 ± 0.37^{bc}
US - 10min		1.49 ± 0.02^{abc}	23.29 ± 2.53^{abc}	91.38 ± 1.65^{abcd}	94.61 ± 0.86^{abc}
US - 20min	45 °C	1.42 ± 0.01^{bc}	30.97 ± 1.37^{a}	93.20 ± 0.61^{abc}	96.00 ± 0.35^{ab}
US - 30min		1.40 ± 0.02^{bc}	28.15 ± 1.05^{a}	93.85 ± 0.41^{a}	97.13 ± 2.28^{a}
US - 40min		1.38 ± 0.01^{c}	25.44 ± 1.13^{ab}	94.51 ± 1.26^{a}	95.98 ± 0.40^{ab}

Means followed by different lowercase letters in the same column represent significant difference by the Tukey test ($p<0.05$).

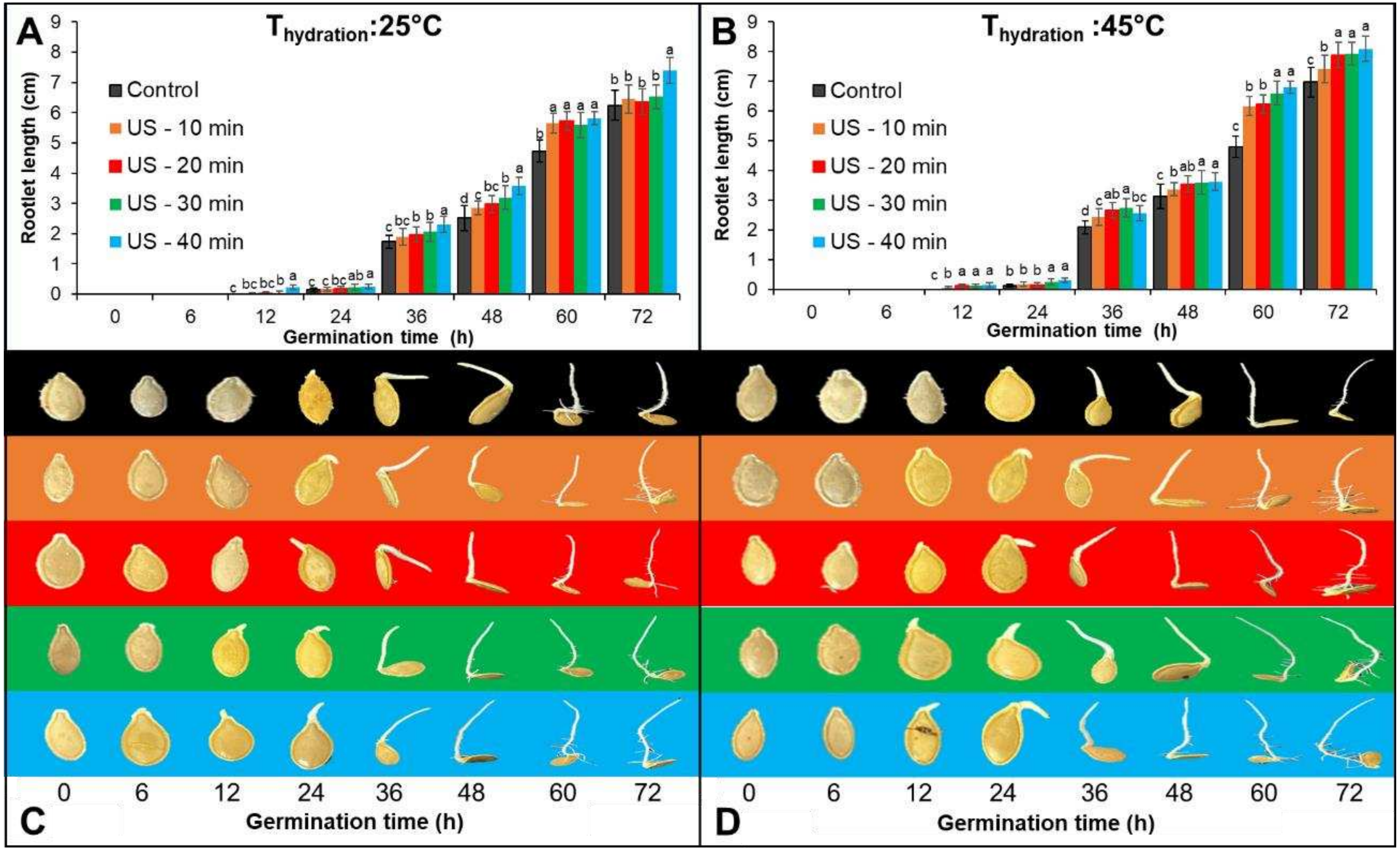

Fig. 4 Impact of ultrasound assisted hydration at 25 °C (A) and 45 °C (B) on the rootlet length during pumpkin seed germination and on the visual aspect (C: $T_{hydration}$: 25°C; D: $T_{hydration}$: 45°C) during 72 hours of germination. Different lowercase at same time and same hydration temperature represents significant difference ($p<0.05$) by the Tukey test among the treatments

In general, the rootlet length was longer for the sonicated samples, regardless of the hydration temperature ($p<0.05$). Such differences are clear in the visual appearance of the seeds (Fig. 4C,D), especially between 12 h and 36 h of germination. According to Miano et al. (2016), the sonication during seeds hydration results in (i) improvements on mass transfer, which facilitates the transport of water and nutrients, and (ii) activation of enzymes responsible for germination. Furthermore, ultrasound can stimulate the production of endogenous hormones, such as auxin, which regulates cell division and elongation (Sun et al., 2020). This hormonal stimulus explains the positive effect of ultrasound on root growth (Ampofo & Ngadi, 2020).

After the total germination time (72 h), for samples hydrated at 25 °C, the greatest length was observed for the sample sonicated for 4 cycles of 10 min (7.4 cm, increase of 19.4% compared to the control sample – 6.2 cm), while for samples hydrated at 45 °C the longest length was observed in samples with 2, 3 and 4 cycles of 10 min US (7.9, 7.9 and 8.1 cm, an increase of up to 15.7% compared to the control sample – 7.0 cm) ($p<0.05$).

The cavitation induced by US result in structural changes that can increase the water absorption by the embryo (Yaldagard et al., 2008). In this way, these factors can contribute to the embryo and radicle development. However, it is worth highlighting that at 25 °C, 4 sonication cycles of 10 min were necessary to reach this effect, while at 45 °C, two cycles were sufficient, showing a combined effect between temperature and sonication in the process.

Similar behaviors have already been observed for other vegetables in the literature; Ampofo and Ngadi (2020) found that after 96 h of germination, the ultrasonic treatment with greater intensity (360 W; 60 min) increased by up to 16.9% and 72.4% the radicle length of common bean (*Phaseolus vulgaris*), when compared to the US treatment with lower intensity (180 W; 60 min) and the control, respectively. In another study, Chiu & Sung (2014) observed that the rootlet length of pea seeds treated with the US (7.47 cm) was 502.4% greater than the control sample (1.24 cm). Similarly, Carvalho et al. (2024) demonstrated that US also promoted improvements in the root length of barley grains. Whereas the size of untreated grains remained relatively stable throughout germination (0.85–0.90 cm), samples subjected to US exhibited significant root growth, with an increase of up to 55.6%, reaching ~1.40 cm (Carvalho et al., 2024).

3.3. Impact of US-assisted hydration on the proximate composition, reducing sugar content, total phenolics and soluble protein of germinated pumpkin seed flours

Table 3 presents the proximate composition of non-germinated pumpkin seed flour and the flours obtained after drying the germinated seeds that were hydrated in a thermostatic bath at 25 or 45 ºC (controls) or hydrated at the same temperatures, but with part of the time subjected to ultrasound pulses (1 to 4 pulses of 10 min). Moisture, protein, lipid, and carbohydrate content were affected by the number of sonication cycles during the seed hydration process, at both temperatures evaluated ($p<0.05$). However, for the ash content, no significant difference was observed ($p>0.05$).

The total protein content increased up to 22.8% and 28.2% (4 sonication cycles of 10 min at 45 °C) compared to the germinated control flour and the non-germinated sample, respectively ($p<0.05$). This result becomes interesting, as protein-rich flour can be added to other food products, improving their nutritional quality and technological properties. The lipid concentration ranged from 35.9% to 40.0%, with an increase ($p<0.05$) of up to 8.4% and 11.4% for the sample sonicated for 4 cycles of 10 min at 45 °C compared to the control sample (25 °C) and non-germinated sample, respectively. Previous studies also observed an increase in protein and lipid contents of different seeds after germination (Pilco-Quesada et al., 2020; Atudorei et al., 2021), as well as in combination with ultrasound followed by germination (Naumenko et al., 2022).

This increase in protein and lipid content may be related to the synthesis of these compounds during germination (Liu et al., 2021), as well as the reduction in the dry weight of germinated seeds, explained especially by the loss of carbohydrate mass through respiration, metabolism, and seed growth (Sharma et al., 2017). Previous results obtained with germinated seeds show that during root development, carbohydrates are metabolized into smaller molecules, releasing energy for synthesizing other molecules, such as proteins and lipids (Zhang et al., 2020; Liu et al., 2021). Corroborating this hypothesis, in the present work, a decrease in the total carbohydrate content of germinated seed flours was observed ($p<0.05$), especially for those sonicated for up to 4 cycles of 10 min (reduction of up to 27.9%) (Table 3). The enhancing effect of sonication is also reported in other studies (Yang et al., 2015; Liu et al., 2021) and can be explained by the increased availability and action of enzymes during germination.

Table 3 Impact of ultrasound assisted hydration at 25°C and 45°C on the proximate composition of germinated pumpkin seed flour

Sample	Hydration temperature	Moisture (% - WM)	Protein (% - WM)	Lipid (% - WM)	Total carbohydrates (% - WM)	Ash (% - WM)
Non-hydrated and non-germinated sample		4.00 ± 0.00^{a}	22.8 ± 0.2^{f}	35.9 ± 0.1^{g}	33.7 ± 0.3^{a}	3.65 ± 0.08^{a}
Control		3.90 ± 0.07^{b}	25.3 ± 0.4^{e}	36.9 ± 0.6^{fg}	30.2 ± 1.0^{b}	3.64 ± 0.09^{a}
US - 10min		3.90 ± 0.04^{ab}	26.2 ± 0.4^{d}	37.5 ± 0.6^{def}	28.7 ± 0.7^{cd}	3.65 ± 0.08^{a}
US - 20min	25 °C	3.87 ± 0.02^{b}	26.5 ± 0.3^{d}	38.2 ± 0.8^{cde}	27.8 ± 0.8^{de}	3.63 ± 0.06^{a}
US - 30min		3.93 ± 0.05^{ab}	27.1 ± 0.3^{bc}	38.9 ± 0.6^{abc}	26.5 ± 0.5^{fg}	3.63 ± 0.08^{a}
US - 40min		3.91 ± 0.07^{ab}	27.6 ± 0.3^{b}	39.1 ± 0.4^{abc}	25.7 ± 0.6^{g}	3.63 ± 0.06^{a}
Control		3.88 ± 0.01^{b}	25.5 ± 0.2^{e}	37.1 ± 0.6^{ef}	29.9 ± 0.4^{bc}	3.65 ± 0.09^{a}
US - 10min		3.90 ± 0.05^{b}	26.2 ± 0.2^{d}	37.8 ± 0.5^{def}	28.5 ± 0.6^{de}	3.63 ± 0.09^{a}
US - 20min	45 °C	3.83 ± 0.08^{b}	26.8 ± 0.2^{cd}	38.4 ± 0.6^{bcd}	27.4 ± 0.5^{ef}	3.61 ± 0.07^{a}
US - 30min		3.84 ± 0.02^{b}	27.3 ± 0.3^{bc}	39.3 ± 0.6^{ab}	25.9 ± 0.6^{g}	3.64 ± 0.08^{a}
US - 40min		3.88 ± 0.08^{b}	28.2 ± 0.2^{a}	40.0 ± 0.7^{a}	24.3 ± 0.7^{h}	3.63 ± 0.08^{a}

Means followed by different lowercase letters in the same column represent significant difference by the Tukey test ($p<0.05$). WM: wet matter

Fig. 5 presents the results regarding the impact of ultrasound-assisted hydration at 25 °C and 45 °C on the concentration of reducing sugars (A), total phenolic content (B), and soluble protein content (C) in pumpkin seed flour germinated at 25 °C for 72 h.

Based on the results, it was observed that both isolated germination and the application of ultrasound during seed hydration promoted an increase in all components evaluated compared to the non-germinated sample ($p<0.05$) (Fig. 5). Specifically, for the reducing sugar content, higher concentrations of this component were observed for samples sonicated for 2 cycles of 10 min both at 25 °C (increase of 326% and 79% compared to the non-germinated sample and germinated sample without the US, respectively) and at 45°C (increase of 304% and 69.7% compared to the non-germinated sample and germinated sample without the US, respectively) (Fig. 5A).

According to Yang et al. (2015), the increase in glucose content may be related to the action of starch hydrolytic enzymes during germination, resulting in the accumulation of glucose in germinated flours (Farzaneh et al., 2017). In this context, US-assisted hydration may have contributed to substrate modification, activation of enzymes, increased solubility of substances, and stimulation of seed metabolism. This finding holds significance for the industry as it has the potential to enhance the flavor profile of flour, rendering it more appealing for food production, particularly in the development of plant-based fermented beverages (Singh et al., 2015).

Regarding the total phenolic compounds (Fig. 5B), the germination process at 25 °C and 45 °C increased the concentration of these compounds by 30.7% and 61.8%, respectively, compared to the flour obtained from non-germinated seeds ($p<0.05$). Regarding the impact of the US, an increase in the concentration of total phenolics was observed for the sonicated samples (notably for the samples sonicated for 2 cycles of 10 min at 25 °C and 45 °C) compared to the non-germinated sample (increase of up to 135.9%) and the non-sonicated germinated samples (increase of up to 81%) ($p<0.05$).

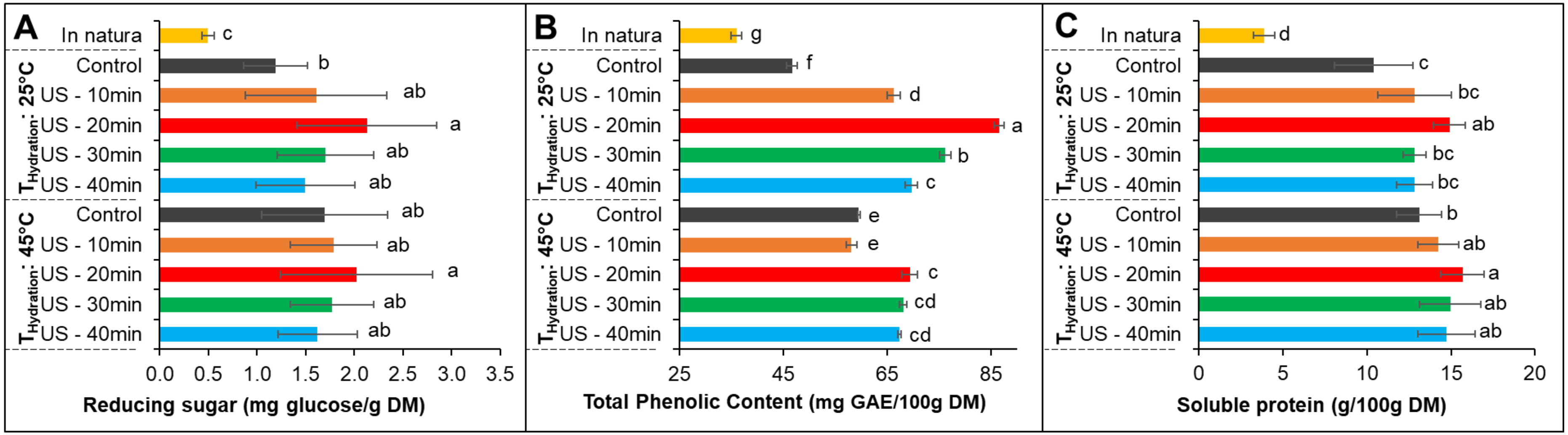

Fig. 5 Impact of ultrasound assisted hydration at 25 °C and 45 °C on the reducing sugars concentration (A), total phenolic content (B) and (C) soluble protein in germinated pumpkin seed flour. The reducing sugars concentration was expressed as mg of glucose per g of dry matter (mg glucose/g DM). TPC was expressed as mg gallic acid equivalents (GAE) per 100 g of dry matter (DM) - (mg GAE/100g DM). Soluble protein was expressed as g of protein per 100 g of dry matter (g/100g DM). Different lowercase letters indicate significant differences among different treatments by Tukey's test at 5% ($p<0.05$)

Germination induces the biosynthesis and hydrolysis of macronutrients bound to the cell wall (such as proteins, starch, and fibers) due to the action of enzymes, such as proteases and amylases, resulting in the formation of smaller compounds that may have biological activities, such as phenolics (Xiang et al., 2023). Furthermore, sonication can act as an abiotic stressor for plants (Pesti-Asbóth et al., 2022), wherein the US treatment triggers the defense response system and stimulates the production of secondary metabolites, such as phenolic compounds (Ding et al., 2019). From an industrial point of view, these data are interesting, as phenolic compounds have been gaining increasing attention for their potential exploitation as dietary supplements, as well as functional ingredients in foods, due to various beneficial properties, including antioxidant and antimicrobial activity (Silva & Pogačnik, 2020).

However, it was observed that longer sonication times (3 and 4 cycles of 10 min of US at 25 °C) resulted in a decrease in the phenolic content compared to the sample with shorter sonication time (2 cycles of 10 minutes of US at 25 °C) ($p<0.05$) (Fig. 5B). This can be explained by the increased exposure of these compounds to environmental conditions during the ultrasonic process (Xiang et al., 2023), emphasizing the importance of careful evaluation of the sonication time and/or number of cycles.

Finally, as observed in Fig. 5C, the germination process at both temperatures increased the soluble protein content (increase of 166.7% for the sample germinated at 25 °C and 235.9% for the sample germinated at 45 °C) compared to the non-germinated sample ($p<0.05$). Furthermore, sonication enhanced the concentration of soluble proteins, with a notable increase observed in the sample subjected to sonication for 2 cycles of 10 min ($p<0.05$). Specifically, the maximum content of soluble protein in the flours was 15.7 ± 1.3 g/100g DM (for sample hydrated at 45 °C and using 2 cycles of US), resulting in increases of up to 51.0%, 19.8%, and 304.4% compared to samples germinated at 25 °C and 45 °C without sonication intervention and the non-germinated sample, respectively ($p<0.05$). The increase in soluble protein content during seed germination with sonication assistance may occur due to the action of proteolytic enzymes on proteins, which may be enhanced by greater release during sonication, resulting in the generation of soluble fractions (peptides and amino acids), potentially improving the bioavailability of this nutrient (Zhang et al., 2020).

Additionally, these results are relevant for the food industry, as the increase in protein solubility is important during food production, affecting different quality characteristics such as appearance, viscosity, flavor, and stability (Cosson et al., 2022) – for example, potentially allowing the production of vegetable alternatives for milk.

Based on the results presented in this work, it is concluded that US technology is an advantageous strategy for hydration and germination of pumpkin seeds, with positive impacts on the quality of the flour. Specifically, the processes with 4 cycles of 10 min of sonication stand out to enhance the kinetics of hydration and germination, as well as increasing the concentration of proteins and lipids in the flour obtained from germinated pumpkin seeds; and the process with 2 cycles of sonication to increase the content of nutrients such as reducing sugars, phenolic compounds, and soluble proteins. These results emphasize that the ideal process condition may vary depending on the specific application, leaving the industry responsible for selecting the one that best suits its needs. In parallel, prolonged germination times lead to higher process costs and favor the growth of undesirable microorganisms. Thus, the data observed in this study are of great importance for understanding the germination process of pumpkin seeds and elucidating the impact of ultrasound during these processes, as well as on the quality of the obtained flour.

Therefore, the germination and sonication, as well as the optimization of these processes, become of great importance in development of value-added products in the food industry. Germinated seeds can be used as culinary ingredients or in development of plant-based protein foods, such as meat analogs, beverages, fermented beverages, bakery, and pasta (Tan et al., 2023).

Despite the important results obtained in this work, it does not completely fill the knowledge gap regarding the impact of sonication and germination on the quality of flour produced from pumpkin seeds. In future research, it is essential to better understand the effects of germination on the flavor and nutritional profile of products obtained from germinated seeds (Ebert, 2022), since several biochemical reactions that occur during this process can positively influence these characteristics of foods.

Regarding sensory aspects, it is expected a bitterness reduction, due to the conversion of phytates and phenolics into no bitter compounds, and a sweetness increase, due to partial starch hydrolysis by amylases with the release of mono- and disaccharides (Andressa et al., 2023a). Both changes tend to make the final product more palatable (Shen, 2024). With respect to the nutritional profile, germination can increase the availability of vitamins, minerals, and antioxidants compounds (do Nascimento et al., 2022). Furthermore, this process can reduce the levels of antinutrients, such as phytates, promoting greater minerals bioavailability (do Nascimento et al., 2022).

4. Conclusion

This study demonstrated, for the first time, that ultrasound technology applied in pulses during the hydration stage of pumpkin seeds could enhance the kinetics of hydration, germination, and the quality of the obtained flours. The time required for hydration was reduced by up to 54.3% (sample with 4 cycles of 10 min of sonication at 45 °C), while the germination time was reduced by up to 11.7% (sample with 4 cycles of 10 min of sonication at 25 °C). These occurrences had positive impacts on the quality of germinated flour, with increases observed in protein (up to 22.8 and 28.2%) and lipid contents (up to 8.4% and 11.4%) for sonicated samples (especially the sample sonicated for 4 cycles of 10 min at 45 °C) compared to the non-US geminated sample and non-germinated sample, respectively. Furthermore, the effect of US pulses on hydration, especially for the sample with 2 cycles of 10 min, favored an increase in the levels of reducing sugars (up to 79%), total phenolic compounds (up to 81%) and soluble proteins (up to 19.8%) compared to the germinated sample without US intervention. Therefore, the combination of ultrasound technology applied in pulses during seed hydration associated with germination proved to be effective in enhancing the reuse of pumpkin seeds, aiming to obtain a functional ingredient for the preparation of new products, such as meat analogs, beverages, fermented beverages, bakery, and pasta.

5. References

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Chapter 3. Impact of ultrasound-assisted intermittent hydration during pumpkin seed germination on the structure, nutritional, bioactive, physical and techno-functional properties of flours

Abstract

This study investigated the ultrasound (US)-assisted intermittent hydration (2 cycles of 10 min, 38 W/L, 25 kHz) at two temperatures (25 or 45°C) and germination (3 days, 25 °C) of pumpkin seeds, evaluating the structure, nutritional, bioactive, color, and techno-functional properties of the obtained flours. US-assisted hydration resulted in a lighter flour and a more porous and dispersed structure, which was intensified by the increase in hydration temperature. Germination promoted changes in flour's aliphatic hydrocarbons, amino acids and carbohydrates, especially in the C-H and C-O bonds, as evidenced by FTIR. In addition, germination and sonication decreased the starch crystallinity degree (≤14.3). US treatment did not affect the flours' mineral nor fatty acid profile. On the other hand, sonication was efficient in increasing the content of GABA (15.6% and 13.7%), ABTS (29.4% and 9.7%) and DPPH antioxidant activities (40.8% and 2.4%) compared to non-sonicated flours at 25 °C and 45 °C, respectively. Regarding techno-functional properties, US increased the water retention capacity, solubility index, foam capacity and foam stability by up to 49%, 68%, 143% and 208%, respectively. The positive effects of sonication, particularly at 25 °C, on the nutritional and techno-functional properties of germinated pumpkin seed flour highlight its potential as a sustainable alternative for developing innovative food ingredients, with possible applications in products such as breads, cakes, cookies, and beverages.

Keywords: Emerging technologies; sonication; clean-label ingredient; sustainability; pumpkin seeds flour; agro-industrial by products; valorization

1. Introduction

The food industry generates substantial quantities of waste during vegetable processing, without utilizing its full potential (Massironi et al., 2023). Sustainable agricultural practices aim to minimize waste and maximize resource efficiency, promoting a circular economy, such as valorizing by-products by converting them into valuable products (Santos et al., 2024).

Pumpkin seed, a by-product of pumpkin (*Curcubita mochata*) processing, is a potential functional ingredient for the food industry (Syed et al., 2010; Syed et al., 2019) due to its nutritional and techno-functional properties (Massironi et al., 2022). This seed is rich in phenolic compounds, antioxidants, proteins (~25% dry basis), lipids (~36% dry basis), and minerals (~4% dry basis) (Andjelkovic et al., 2010; Pacheco et al., 2024a).

Germination is a bioprocessing method that can improve the nutritional, physical and chemical quality of seeds (Miyahira et al., 2021; Andressa et al., 2024), potentially converting them into functional clean-label ingredients (Polachini et al., 2023). However, this process presents several challenges, such as the long hydration (often taking up to 48 hours) (Kalita et al., 2021) and germination times (>72 hours), making the process slow, costly, and increasing the risk of microbial contamination (Ding & Feng, 2018). To overcome these challenges, new strategies are being developed, including the use of emerging technologies, such as ultrasound, to accelerate and improve both the hydration and germination through heat transfer (Guimarães et al., 2020, Pacheco et al., 2024b).

In fact, our previous study (Pacheco et al., 2024b) focused primarily on evaluating the effect of ultrasound-assisted hydration and its consequences on the germination kinetics and proximate composition of germinated pumpkin seeds. However, essential aspects related to structural, techno-functional, and bioactive properties, which are fundamental for determining the potential applications of these flours in the food industry, were not addressed. To fill this gap, in the present study, the most efficient ultrasound-assisted hydration treatments (2 cycles of 10 minutes at 25 and 45°C) were selected, and the structural modifications (SEM, fluorescence spectroscopy, FTIR, XRD), as well as the nutritional, bioactive (fatty acid profile, minerals, GABA, antioxidant activity), and techno-functional properties (water and oil holding capacities, foaming capacity and stability, swelling and solubility indices), along with the color parameters of the flours, were evaluated. This in-depth analysis is essential to understand the impact of sonication on the structural modifications of germinated pumpkin seed flour and to identify the best industrial applications for these flours, making this study fundamental for the development of a sustainable, clean-label, and high-added-value ingredient for the food industry.

2. Materials and Methods

2.1. Ultrasound-assisted hydration, germination process and flour production

Pumpkin seeds (*Cucurbita moschata*) were obtained from the company Doces Mirahy (Miraí, Minas Gerais Brazil), as a by-product from the pulp. After receiving, the seeds were dried at 45 °C for 24 h, vacuum packed and stored at 7 °C for up to 24 h.

For intermittent ultrasound-assisted hydration, approximately 40 g of pumpkin seeds (wet basis) were sanitized with 200 mg.L^{-1} sodium hypochlorite solution for 15 min. After sanitization, US-assisted hydration was performed using 2 cycles of 10 min in an ultrasonic bath (Unique, model USC 2800 A, Brazil) with temperature control, operating at a frequency of 25 kHz, nominal power of 450 W, and volumetric power of 38 W/L, interspersed with 50 min in a thermostatic bath (SSDu 10L, Solidsteel, Piracicaba, Brazil) at controlled temperatures of 25 ± 2 °C and 45 ± 2 °C. The first sonication cycle was carried out at the beginning of hydration. After US cycles, the hydration was completed in the thermostatic bath until reaching a humidity of 45% (0.82 g of water/g of dry matter). These conditions were chosen based on the best results for hydration (25 °C and 45 °C) and germination (25 °C) as determined by Pacheco et al. (2024b). The conventional hydration (non-ultrasound-assisted hydration) was carried out under the same temperature conditions in a thermostatic bath until reaching the same moisture.

After hydration, the seeds underwent a germination process. For this, 40 g of seeds from each treatment were incubated in a BOD (Novaética, Rio de Janeiro, Brazil) at a controlled temperature of 25 °C for 72 h in the dark. To maintain optimal relative humidity (80-85%), seeds were sprayed with distilled water three times a day.

After germination process, the seeds were weighed and dried in an oven (Q819V2, QUIMIS, Diadema, Brazil) at 50 °C for 12 h. After drying, the rootlets were removed from the germinated seeds, and the germinated and non-germinated seeds were ground in an analytical mill (model IKA® A11 B S32, 180 W). Subsequently, the flours were packaged in vacuum-sealed plastic bags and stored at 7 °C for later analysis. Germinated samples, treated or not with ultrasound, were identified in the text as no-US and US-20 min (25 °C and 45 °C), respectively, while the control sample as non-germinated, totalizing 5 treatments. Figure 1 shows the flow of obtaining flour and its proximate composition.

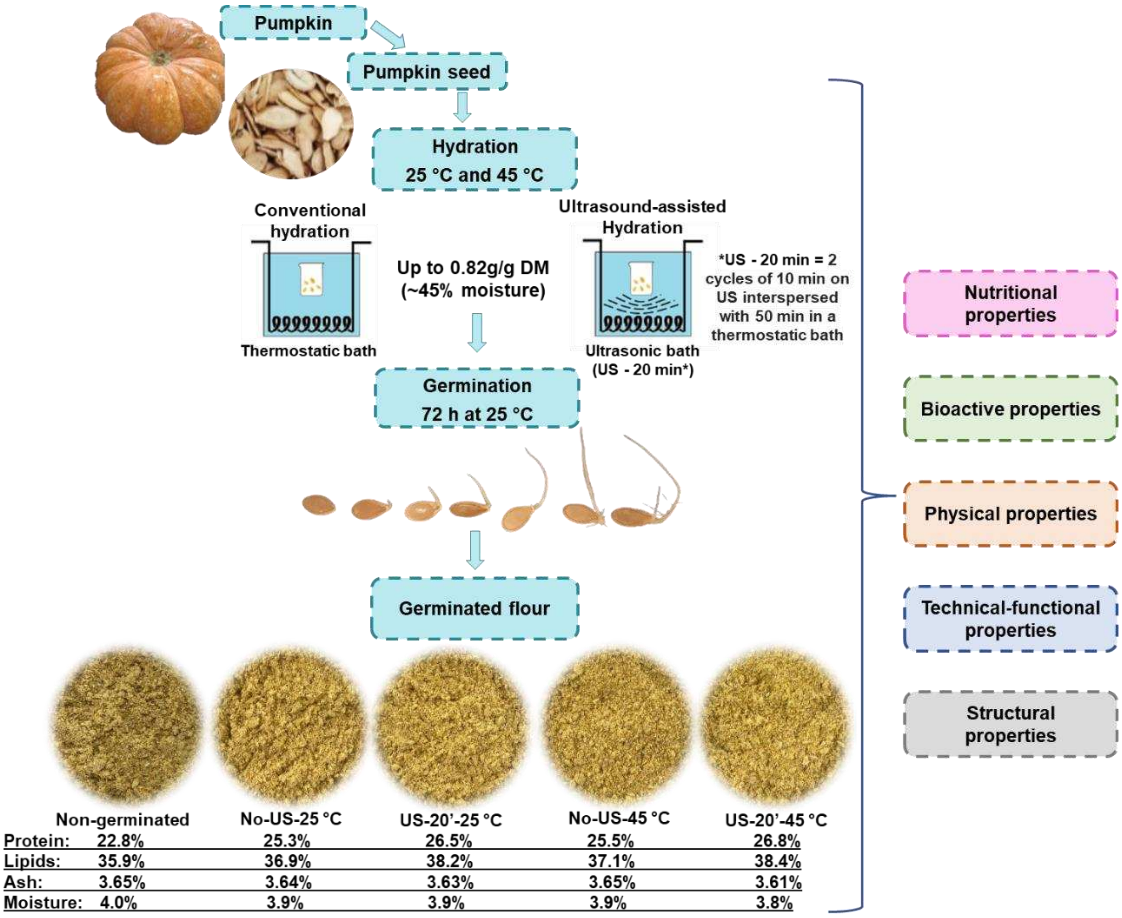

Figure 1. Experimental planning.

2.2. Structural properties

2.2.1. Scanning electron microscopy (SEM)

SEM was conducted using the method of Adebo et al. (2018). Flour samples were initially spray coated with carbon (Quorum Q150TE, Quorum Technologies, Lewes, UK), then electron beamed and visualized using a scanning electron microscope (Vega 3 XMU, TESCAN, Brno, Czech Republic) under vacuum. The microscope was operated at an accelerating voltage of 10 kV, and images were captured at a magnification of ×1000.

2.2.2. Intrinsic Fluorescence Spectroscopy

The intrinsic fluorescence of the seed flours was determined using the methodology described by Sosa et al. (2019) using a SpectraMax M5 fluorescence spectrophotometer (Molecular Devices, San Diego, USA), with modifications. For this, 25 mg of flour was dissolved in 5 mL of 50 mM acetic acid solution at room temperature for 60 min under stirring and then centrifuged (10,000 × g, 15 min, 25 °C). Then, 300 µL of the supernatant was excited at an excitation wavelength of 290 nm, and the emission spectra were recorded in the range of 300 - 420 nm with a slit of 2 nm.

2.2.3. Fourier transform infrared (FTIR) spectroscopic analysis

Germinated and non-germinated pumpkin seed flour were analyzed using a Fourier transform infrared spectrophotometer, Cary 630 FTIR (Agilent Technologies, California, USA). The absorbance range used was 4000 to 500 cm^{-1}, with a resolution of 4 cm^{-1} and 8 scans (Gunarathne et al., 2022). Representative wavenumbers (cm^{-1}) of non-germinated pumpkin seed flour were selected according to the literature. The percentage relative change (PRC) in intensity was calculated as described in Equation 1 (1). A negative value indicates increased intensity (Chinma et al., 2022).

$$\mathrm{PRC} = \frac{raw\ length - germinated\ length}{raw\ length}\ x\ 100\ (1)$$

2.2.4. X-ray diffaction (XRD) analysis

The crystallographic structure was determined using an X-ray diffractometer (XRD-6000, Shanghai Drawel Scientific Instrument Co., Ltd., Japan) equipped with a divergence slit, using monochromatic CuK radiation (λ = 0.15406 nm - 40 kV and 30 mA) at a scan rate of 2.0 deg.min^{-1} covering the 2θ range from 10 to 80°. The relative crystallinity (RC) was calculated using the areas of the crystalline and amorphous regions in the X-ray diffractogram (Equation 2 (2)). The d-spacing were calculated using the Bragg's Equation 3 (3).

$$\mathrm{RC} = \frac{\text{crystalline area}}{total\ area} \quad (2)$$

$$n\lambda = 2 * d * \sin\theta \quad (3)$$

where *n* is an integer; *λ* is the wavelength of incident wavelength; *d* is the spacing between the planes in the atomic lattice, *θ* is the Bragg's angle in radian (peak center).

2.3. Composition, bioactive and physical-chemical properties

2.3.1. Fatty acid profile

The lipid fraction of non-germinated and germinated pumpkin seed flours was extracted from 1 g of sample using a mixture of methanol, chloroform, and water, according to the method described by Bligh and Dyer (1959). Subsequently, the lipids were derivatized using the method of Hartman and Lago (1973). Then, the fatty acid methyl esters (FAME) dissolved in hexane were analyzed on a gas chromatograph equipped with a flame ionization detector (GC-FID) (Agilent Technologies, model 7820A, USA). The results were expressed as a percentage of the total area of the chromatograms, considering the FID correction factors and the conversion of esters into acids (Visentainer, 2012).

2.3.2. Mineral profile

The mineral profile of germinated and non-germinated flours was determined by energy-dispersive X-ray fluorescence spectrometry (EDXRF -

model EDX 720, Shimadzu®, Kyoto, Japan, with the software PCEDX 1.11). The equipment operated at voltages of 0-40 KeV (Ti-U) and 0-20 KeV (Na-Sc) for 100 seconds each. The results were expressed in mg/100 g on a dry basis.

2.3.3. Gamma-aminobutyric acid (GABA)

The extraction, derivatization, and quantification processes of GABA from pumpkin seed flours were obtained as described by Nascimento et al. (2024). GABA quantification was performed on a 1260 Infinity high-performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA, USA), using γ -aminobutyric acid (Merk, purity >99%) as standard within a linear range of six points (36.8 to 184 mg/L; area = 29.245 × concentration of γ-aminobutyric acid + 30.702; r^2 = 0.9994). The injection volume was 10 µL, and detection was performed at 254 nm. The results were expressed in mg of GABA per 100 g of sample (on a dry basis for flours).

2.3.4. Antioxidant activity

The flour antioxidant activity was evaluated by both DPPH and ABTS methods, after extraction of 1 g of flour by 20 mL of 80% methanol under ultrasound bath (Planatec, São Paulo, Brazil; 40 kHz, 100 W) for 5 min at 25 °C. The samples were centrifuged (Sorvall Legend Micro 17R-Thermoscirntfic, USA) at 5000 x g for 5 min, and the supernatant was retained.

The antioxidant activity using the DPPH method followed the procedure described by Bubelová et al. (2018). A 100 µL of the methanolic extract of the sample was mixed with 3.9 mL of methanolic DPPH solution. The mixture was then incubated in the dark for 60 min, and the absorbance was measured at 515 nm. The antioxidant activity was calculated using a calibration curve obtained with Trolox (standard curve equation: [Trolox] = 153.81 × $Abs_{at485nm}$ - 3.01; r^2 = 0.9904), and the results were expressed as mg Trolox/ 100 g pumpkin seed flour (on a dry basis for flours).

Antioxidant activity using ABTS was performed according to Re et al. (1999), with minor modifications. An ABTS+ stock solution was prepared by mixing a seven mM ABTS solution in 2.45 mM potassium persulfate in a 1:1 ratio. After this, the mixture was left to rest in the dark, at room temperature, for 16 hours before use. Then, the ABTS+ stock solution was diluted with ethanol until its absorbance reached approximately 0.70 ± 0.05. The mixture consisted of 50 µL of sample and 3.5 mL of ABTS+ stock solution and was incubated in the dark for 60 minutes, and absorbance was subsequently measured at 734 nm. Antioxidant activity was determined using the same calibration curve obtained with Trolox, and the results were expressed in mg Trolox/ 100 g of pumpkin seed flour (on a dry basis for flours).

2.3.5. Color parameters

The instrumental color of non-germinated and germinated pumpkin seed flours was determined by reading on a Konica Minolta CR10 colorimeter (Minolta, Chiyoda, Japan). Color measurements were expressed in terms of luminosity L*, and the chromaticity parameters a* and b*. The total color difference (ΔE) was used in relation to the non-germinated flour sample as a reference and was determined according to (Equation 4 (4)).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

2.4. Techno-functional properties

2.4.1. Water and oil holding capacities

To determine the water holding capacity (WHC), 2 g of flour and 30 mL of distilled water were mixed in a 50 mL centrifuge tube. After 2 min, samples were centrifuged at 3500 × g for 30 min at 25 °C (Alfaro-Diaz et al., 2021), drained and the amount of water absorption was determined by the grams of water retained per gram of the sample on a dry basis (Alfaro-Diaz et al., 2021).

Oil holding capacity (OHC) was calculated as the weight of oil absorbed. For this, 10 mL of refined soybean oil was added to 2 g of each flour in a 50 mL centrifuge tube and mixed for 2 min. Then, the mixture was rested at 28 °C for 30 min, centrifuged at 15.000 × g for 20 min (Alfaro-Diaz et al., 2021), drained and amount of oil absorption was determined by the grams of oil retained per gram of the sample on a dry basis (Alfaro-Diaz et al., 2021).

2.4.2. Foaming capacity (FC) and stability (FS)

To analyze the foam's capacity and stability, the method described by Elkhalifa and Bernhardt (2010) was used with modifications. For this, 0.5 g of flour was mixed with 25 mL of distilled water, and the suspension was mixed using a high shear disperser (HSD) at 15,000 rpm for 2 min at 23 °C. The contents were immediately transferred to a 100 mL graduated cylinder, and the foam volume was recorded. Foaming capacity (FC) was expressed as the percentage increase in volume using the Equation 5 (5).

$$\text{FC} = \frac{\text{volume after} - \text{volume before}}{\text{volume before}} \text{ x } 100 \quad (5)$$

The samples were stood for 60 min and the volumes of the foam were recorded again, and the foam stability was calculated as follows (Equation 6 (6)).

$$\text{FS} = \frac{\text{Volume after 60 min of rest}}{\text{Volume at 0 min}} \text{ x } 100 \quad (6)$$

2.4.3. Swelling index and solubility index

The swelling and solubility indices were determined according to the method described by Wani et al. (2013). The sample (0.6 g) was mixed with 20 mL of distilled water in tubes, heated at 90 °C for 30 min in a water bath, and then centrifugated for 15 min at 3,500 × g at 4 °C. The swelling index was determined from the weight gained by the samples after draining the supernatant (Equation 7 (7)). The supernatant containing soluble matter was dried in a static oven, and the residues' weight was expressed as a solubility index (Equation 8 (8)).

$$\text{Swelling Index} = \frac{\text{Swelling capacity of flour (g)}}{\text{Volume of the flour (g)}} \quad (7)$$

$$\text{Solubility Index} = \frac{\text{Residual weight after drying}}{\text{Weigth of the initial sample (g)}} \text{ x } 100 \quad (8)$$

2.5. Experimental design and statistical analysis

The processes and experiments were carried out in three replications, and each experimental unit was carried out in triplicate. The results were expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA, followed by Tukey's post-hoc test, to compare the effects among different treatments with a 95% confidence level using Statistica software, version 9.2 (StatiSoft INC, USA).

3. Results and discussion

3.1. Structural properties

3.1.1. Scanning electron microscopy (SEM)

The effect of germination and ultrasound on the microstructure of pumpkin seed flour was analyzed by SEM, revealing significant differences in the flour structures across different treatments (Figure 2). As expected, the non-hydrated and non-germinated sample exhibited a compact and smooth surface, with no ruptures or significant changes, characteristic of untreated seeds. Small structural alterations began to emerge after germination at 25 °C and 45 °C compared to the non-germinated sample. Initial signs of surface modifications are visible, such as the appearance of small ruptures (red arrow in Figure 2). More substantial changes were observed when hydration was performed with 2 cycles of sonication (US-20 min) at 25 °C and 45 °C. In this case, the seed surface became more porous and showed significant structural breakage (blue arrow in Figure 2) compared to non-germinated and germinated samples without US. These changes indicate cell wall rupture, facilitating water uptake (Xia et al., 2020; Chen et al., 2023). Indeed, the structural damage combined with the increased mass transfer during ultrasound-assisted hydration was responsible for the reduction of up to 54.3% in the hydration time of pumpkin seeds, as reported by Pacheco et al. (2024b).

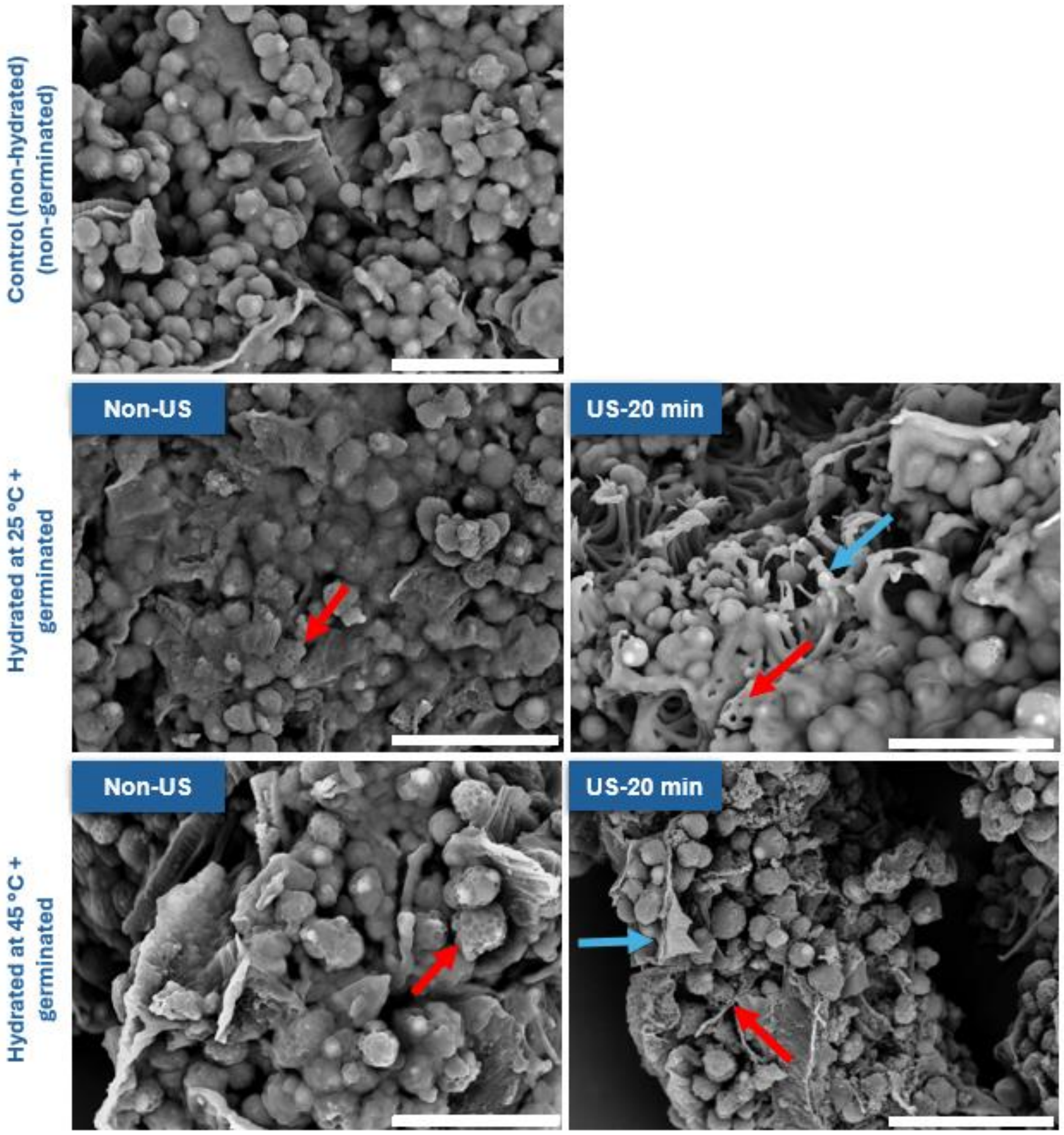

Figure 2. Scanning electron microscopy of non-germinated and germinated pumpkin seed flour without and with ultrasound treatment during hydration at 25 °C and 45 °C. *Bars represent 30 µm*. Red and blue arrows represent structural changes discussed on section 3.1.1.

3.1.2 Intrinsic Fluorescence Spectroscopy (IF)

The fluorescence spectrum, influenced by the polarity of the environment and interactions of tryptophan (Trp) and tyrosine residues, is a sensitive tool for characterizing proteins and their conformations (Pallarés et al., 2004). As shown in Figure 3, the intrinsic fluorescence spectrum of germinated samples exhibited a 38-40% increase in fluorescence intensity at the peak compared to non-germinated flour ($p<0.05$). This suggests a possible exposure of proteins' hydrophobic groups, such as tryptophan, tyrosine, and phenylalanine, induced by enzymatic hydrolysis during germination, which aligns with the observed increase in soluble protein concentration reported in a previous study (Pacheco et al., 2024b). Notably, the combination of ultrasound and temperature (45 °C) during seed hydration led to an even more significant increase in fluorescence intensity, with a 52% rise (US-20 min-45 °C). This finding indicates that ultrasound-assisted hydration at higher temperatures caused protein unfolding, exposing tryptophan residues to the polar microenvironment (Song et al., 2023; Cunha et al., 2024). Additionally, ultrasound may alter the protein's three-dimensional conformation, thereby increasing access to hydrophobic residues (Wang et al., 2024). Thus, the combination of ultrasound and hydration temperature demonstrated a synergistic effect, resulting in a more substantial increase in fluorescence intensity (Yu et al., 2023).

Another relevant observation in the fluorescence emission spectra was a slight red shift in the peak wavelength of the samples subjected to sonication (US-20 min, 25 °C and 45 °C), with a λ_{max} of 360 nm compared to 358 nm in the non-germinated sample (Figure 3). This shift suggests greater exposure of aromatic amino acids, such as tryptophan, to a more water-affine environment, which can potentially improve the techno-functional properties of the flour (Chen et al., 2013).

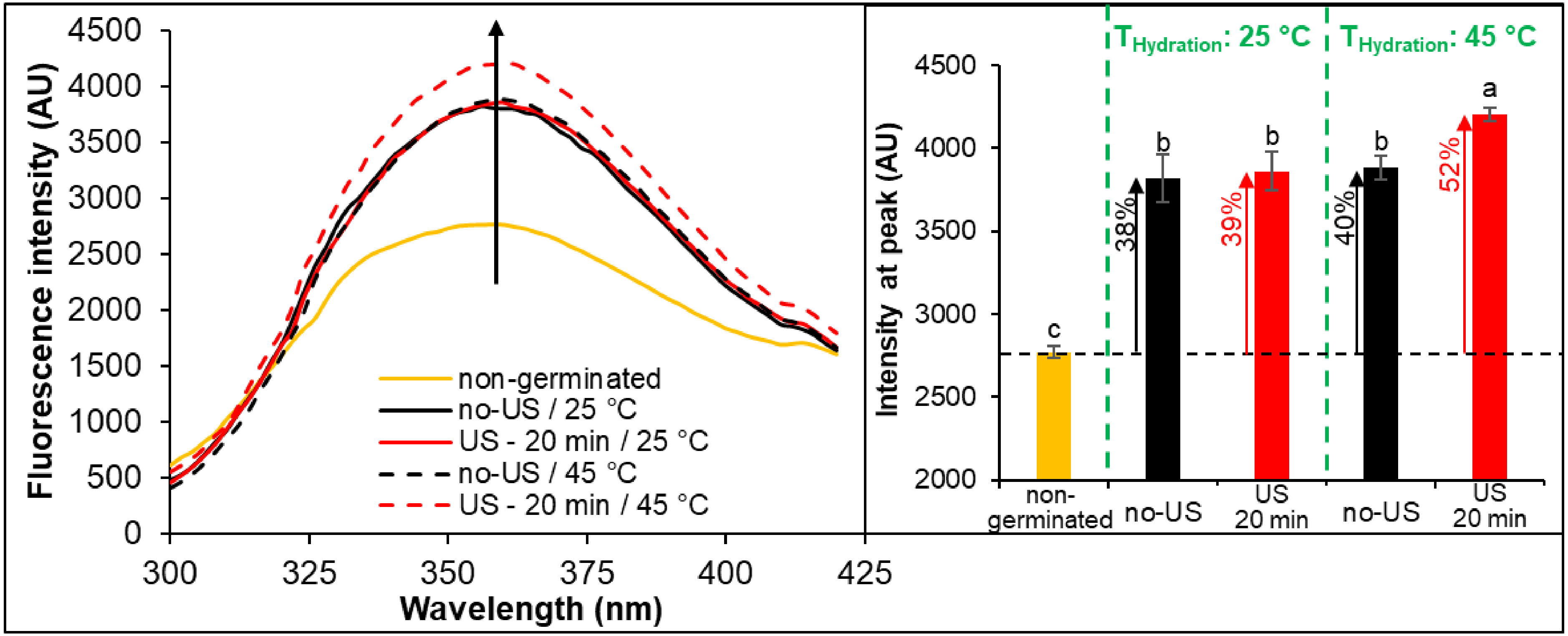

Figure 3. Intrinsic Fluorescence of non-germinated and germinated pumpkin seed flour without and with ultrasound treatment during hydration at 25 °C and 45 °C.

3.1.3. Fourier transform infrared (FTIR) spectroscopic analysis

Non-germinated and germinated pumpkin seed flours were analyzed using FTIR spectroscopy to investigate changes in functional groups (Figure 4). The spectra reveal noticeable changes in various bands at different wavenumbers due to germination and ultrasound-assisted hydration. Percent relative change (PRC) in intensity (Table 1) was used to clarify these changes: a negative PRC indicating an increase in intensity relative to the bands in non-germinated pumpkin seeds, highlighting the impact of germination on functional group intensity (Chinma et al., 2022).

The 1100–900 cm^{-1} region is associated with carbohydrate-related transitions, specifically C–C stretching vibrations (1200–1103 cm^{-1}) and C–O bending vibrations (1047–994 cm^{-1}) (Ying et al., 2017; Vela et al., 2021). The C–O bond elongation peak at 1040.8 cm^{-1} increased with germination and US-assisted hydration ($p<0.05$ – Table 1). According to Vela et al. (2021), the bands at 1022 cm^{-1} and 1047 cm^{-1} are attributed to the amorphous and crystalline structures of starch, respectively, suggesting a possible shift in these structures to the peak at 1040.8 cm^{-1} in the germinated, with or without sonication. The absorbance ratio of 1047/1022 cm^{-1} was highest in the non-germinated sample compared to no-US (25 °C), US-20 min (25 °C), no-US (45 °C), and US-20 min (45 °C) samples, with values of 0.993 versus 0.963, 0.962, 0.973, and 0.964, respectively. These results indicate that germination and sonication promote the transition from highly ordered crystals to disorganized amorphous molecules, a trend also observed by Kaur & Gill (2021). The starch region exhibited evident changes during germination, with a decreasing trend in the 1047/1022 cm^{-1} ratio in germinated flours compared to the control sample (Kaur & Gill, 2021).

The FTIR region from 1100 to 1700 cm^{-1} is associated with proteins, including amide I, amide II, and amide III regions (Yakubu et al., 2022). A decrease in amino acid content was observed at 1236.6 cm^{-1} (C–F stretching of alkyl halides) in the samples hydrated with or without sonication at 45 °C ($p<0.05$). Additionally, an increase in intensity was recorded at 1379.0 cm^{-1} (C–H deformation vibrations) for the not treated with US at 25 °C ($p<0.05$). Changes at this peak suggest an increase in C–H stretching, possibly related to the formation of long-chain linear aliphatic structures (Gunarathne et al., 2022). Higher peaks related to proteins, such as those at 1540.6 cm^{-1} and 1636.1 cm^{-1}, were not affected by germination, ultrasound treatment, nor increased hydration temperature ($p>0.05$).

The FTIR spectra of the flours also reveal other distinct vibrational characteristics. Observed vibrations include O–H stretching from 3700 to 3020 cm^{-1} (up to 3275.8 cm^{-1}) in soluble and non-soluble carbohydrates, C–H stretching from 3020 to 2744 cm^{-1}, and C=O carbonyl group vibrations in lipids prominently observed at 1733 cm^{-1} (Bem et al., 2024). These peaks were also unaffected by germination, ultrasound treatment, and increased hydration temperature. Overall, our results suggest that both treatments reduce the compaction of starch granules and aliphatic hydrocarbons, enhancing water penetration into the starch matrix (Fang et al., 2020).

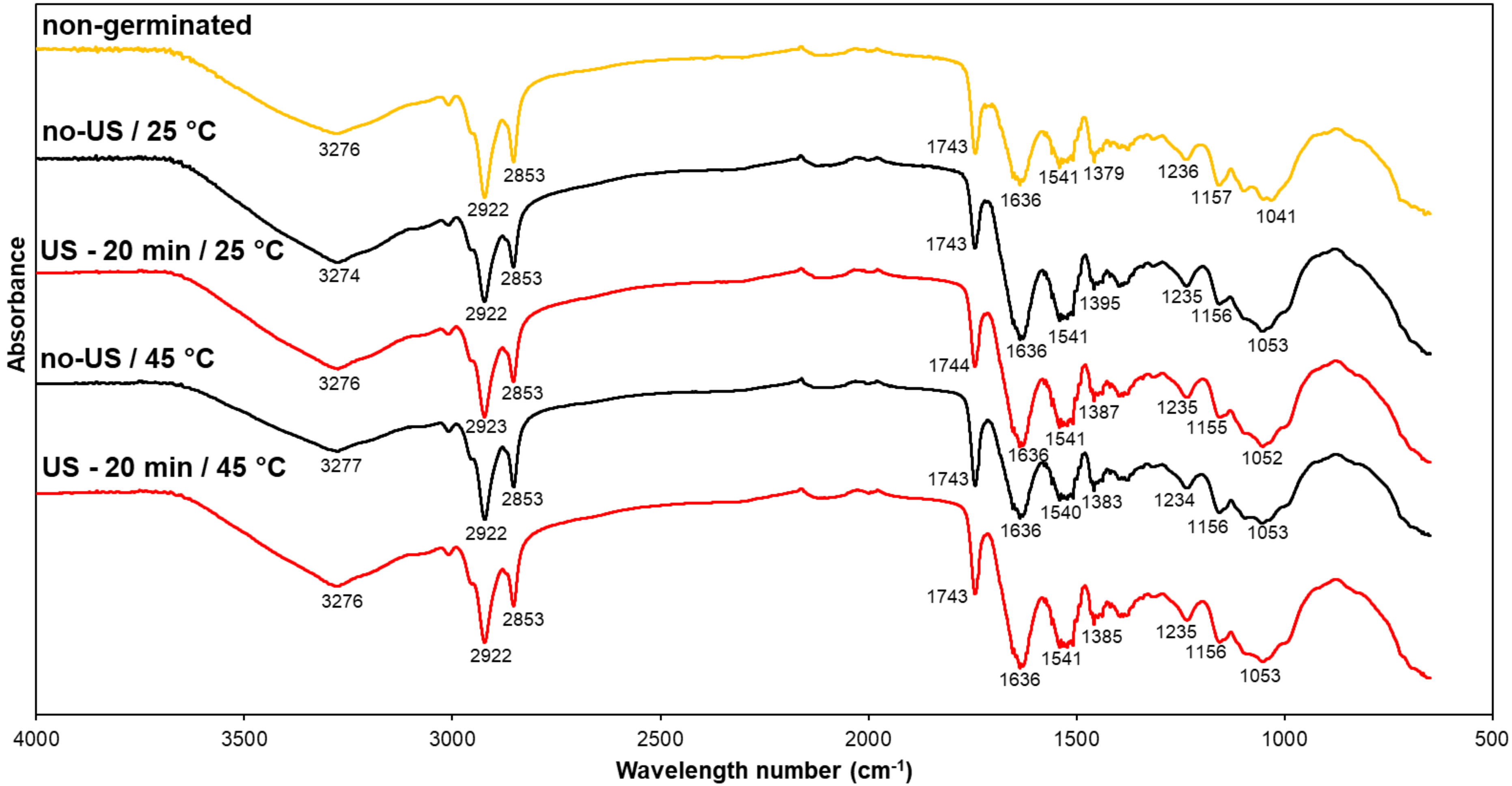

Figure 4. Fourier transfer infrared spectroscopy of non-germinated and germinated pumpkin seed flour without and with ultrasound treatment during hydration at 25 °C and 45 °C.

Table 1. Description and relative change in intensity of FTIR functional groups of non-germinated and ultrasound-pretreated germinated pumpkin seed flours.

Representative wave numbers (cm^{-1}) Reference: non-germinated Sample	Functional group	Probable compound	Relative percentage change in intensity				
			non-germinated	no-US / 25 °C	US - 20 min / 25 °C	no-US / 45 °C	US - 20 min / 45 °C
3275.8	O - H stretching of alcohol	Soluble and non-soluble carbohydrates	0.00 ± 0.04[a]	0.05 ± 0.03[a]	0.00 ± 0.06[a]	-0.04 ± 0.08[a]	-0.01 ± 0.05[a]
2922.5	C – H stretching of alkane	Carboxyl group of triglycerides	0.00 ± 0.03[a]	0.00 ± 0.03[a]	-0.01 ± 0.03[a]	0.01 ± 0.02[a]	0.01 ± 0.02[a]
2853.1	C – H stretching of alkane	Carboxyl group of triglycerides	0.00 ± 0.02[a]	0.00 ± 0.03[a]	-0.01 ± 0.02[a]	0.02 ± 0.03[a]	0.01 ± 0.01[a]
1743.3	C=O Stretching	Lipids	0.00 ± 0.03[a]	-0.01 ± 0.07[a]	-0.03 ± 0.02[a]	-0.01 ± 0.05[a]	0.01 ± 0.04[a]
1636.1	Group C = N/amine stretch/ C = O of oxalic acid	Protein	0.00 ± 0.03[a]	0.01 ± 0.03[a]	-0.01 ± 0.02[a]	0.01 ± 0.04[a]	-0.01 ± 0.04[a]
1540.6	N – O stretching of nitro compounds	Protein	0.00 ± 0.04[a]	0.00 ± 0.04[a]	-0.03 ± 0.03[a]	0.01 ± 0.04[a]	-0.01 ± 0.03[a]
1379.0	C – H deformation vibrations	Aliphatic hydrocarbons	0.00 ± 0.33[b]	-1.20 ± 0.02[a]	-0.62 ± 0.56[ab]	-0.31 ± 0.65[b]	-0.41 ± 0.40[b]
1236.6	C – F stretching of alkyl halide	Amino acids	0.04 ± 0.08[b]	0.10 ± 0.05[ab]	0.12 ± 0.04[ab]	0.19 ± 0.08[a]	0.16 ± 0.03[a]
1157.4	C – 0 – C elongation of antisymmetric bridge	Amino acids	0.00 ± 0.08[a]	0.13 ± 0.13[a]	0.18 ± 0.16[a]	0.08 ± 0.11[a]	0.15 ± 0.10[a]
1040.8	C–O bending vibrations	Carbohydrates	0.00 ± 1.04[b]	-1.13 ± 0.04[a]	-1.12 ± 0.07[a]	-1.21 ± 0.11[a]	-1.14 ± 0.05[a]

Different lowercase letters (line) indicate significant differences between different treatments by Tukey's test at 5% probability ($p<0.05$). References of associated functional groups and probable compounds: Vela et al. (2021); Kaur & Gill (2021); Gunarathne et al. (2022).

3.1.4. X-ray diffraction (XRD)

X-ray diffraction was used to assess the crystallinity of compounds in the non-germinated and germinated pumpkin seed flour samples, especially starch. The peak intensity reflects the ordered crystalline regions, and the electron density differences between the amorphous and crystalline components of the flour (Figure 5). The sharp peaks represent the crystalline regions, and the diffuse peaks represent the amorphous region. The non-germinated and germinated flours showed significant changes in the diffraction peaks (p>0.05), suggesting a shift in the type of crystalline structure due to germination, ultrasound treatment, and increased temperature.

As shown in Table 2, the crystallinity index, diffraction peaks, peak intensity, and D-spacing of all five flour samples ranged from 66.3–77.4%, 20.2–20.9°, 487.3–875.5 and 4.25–4.40 nm, respectively. It can be observed that the crystallinity index for the non-germinated flour sample was higher (77.4 ± 0.73) than for the germinated and sonicated samples (p<0.05). In fact, the relative crystallinity of the flour decreased by up to 14.3%, affecting the complex organization of the starch pattern structures. Similar results were found in barley, groundnut, quinoa and chickpea (Gutiérrez-Osnaya et al., 2020; Chinma et al., 2021; Xing et al., 2021; Sofi et al., 2023).

In addition, a significant shift was observed in the positions of the diffraction peaks between the non-germinated flour (20.9°) and the germinated samples (ranging from 20.2° to 20.4°) (p<0.05). The non-germinated sample also exhibited a smaller d-spacing (4.25 nm) compared to the germinated samples (4.38 and 4.40 nm) (p<0.05). These results suggest that germination altered the crystalline structure of the compounds, particularly starch, leading to a slight increase in the distance between crystalline planes, which was associated with a decrease in crystallinity (Gutiérrez-Cortez et al., 2024).

The germinated samples showed higher peak intensity compared to the non-germinated sample (p<0.05). According to Sofi et al. (2023), germination promotes starch hydrolysis by amylases to provide energy for root growth, which subsequently affects the structure of starch double helices (Li et al., 2017). In the present study, the modification in the distribution of debranched amylopectin chains may account for the observed reduction in crystallinity (Xing et al., 2021).

However, further studies are required to determine whether other factors, such as the extent of branching and molecular weight, also contribute to this decrease (Sun et al., 2015). Additionally, these findings align with the FTIR results, which revealed a shift in the peak at 1040.8 cm^{-1}, suggesting potential changes in the amorphous and crystalline structures of starch in the germinated samples.

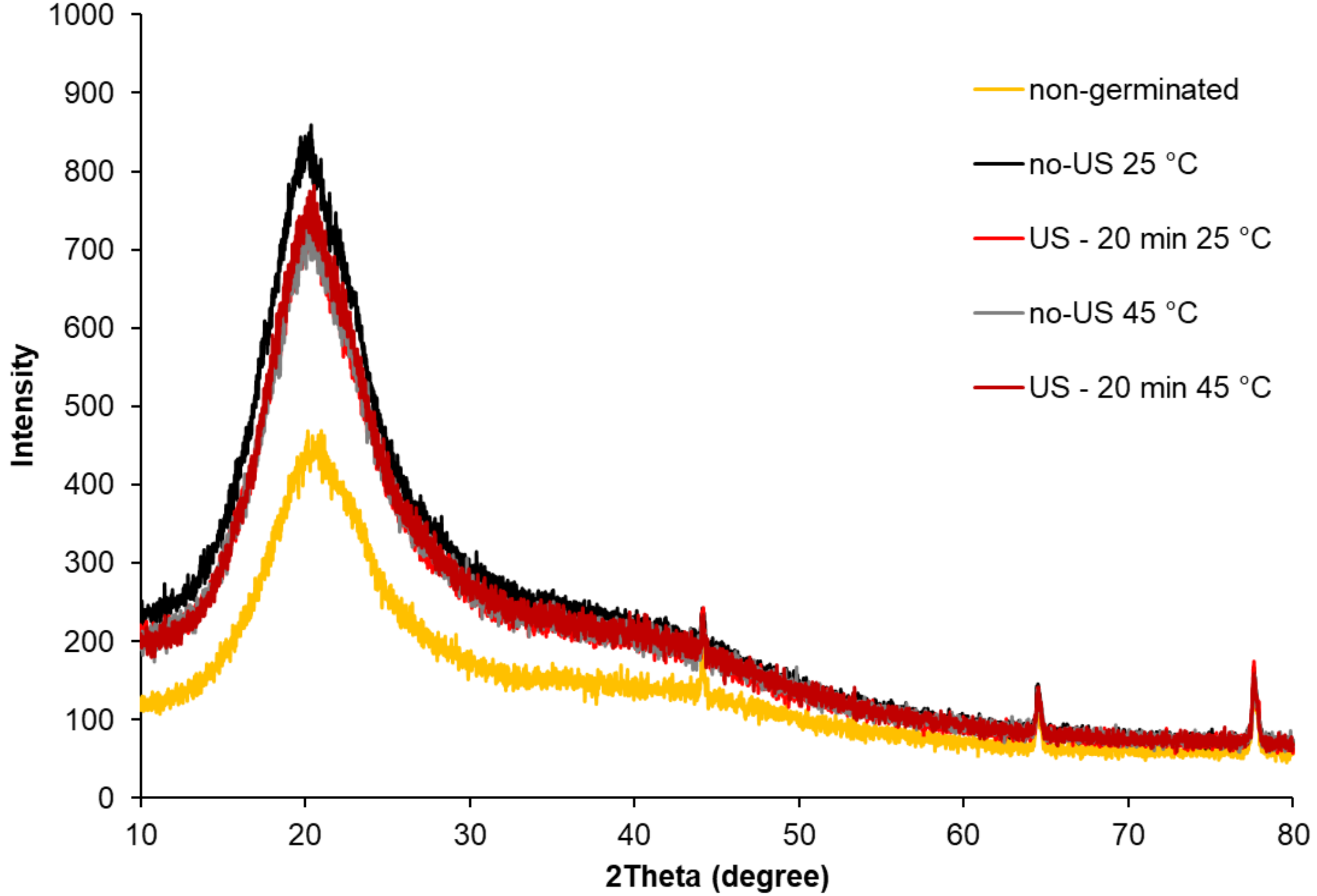

Figure 5. XRD patterns of non-germinated and germinated pumpkin seed flour without and with ultrasound treatment during hydration at 25 °C and 45 °C.

Table 2. XRD parameters of non-germinated and ultrasound-pretreated pumpkin seed flours.

Sample	Crystallinity index (%)	Peak diffraction (°)	Peak intensity	D-spacing (nm)
non-germinated	77.4 ± 0.73^{a}	20.9 ± 0.09^{a}	487.3 ± 29.14^{b}	4.25 ± 0.02^{b}
no-US 25 °C	68.9 ± 0.71^{b}	20.2 ± 0.23^{b}	875.5 ± 41.03^{a}	4.39 ± 0.05^{a}
US - 20 min 25 °C	66.6 ± 0.70^{c}	20.4 ± 0.32^{b}	764.0 ± 56.64^{a}	4.39 ± 0.04^{a}
no-US 45 °C	66.3 ± 0.79^{c}	20.2 ± 0.21^{b}	771.3 ± 94.85^{a}	4.38 ± 0.04^{a}
US - 20 min 45 °C	66.5 ± 0.69^{c}	20.2 ± 0.11^{b}	811.3 ± 86.38^{a}	4.40 ± 0.02^{a}

Different lowercase letters (column) indicate significant differences between different treatments by Tukey's test at 5% probability ($p<0.05$).

3.2. Fatty acid and mineral profile

The fatty acid profiles of non-germinated and germinated pumpkin seed flours are presented in Table 3. The lipids of pumpkin seeds are predominantly composed of polyunsaturated (linoleic, 49.85%), monounsaturated (oleic, 21.07%), and saturated fatty acids (palmitic and stearic, 17.66% and 9.41%, respectively). Overall, the results showed that neither ultrasound-assisted hydration nor germination changed the fatty acids profile in the flour ($p>0.05$). Similarly, Ahmed et al. (2024) observed that the fatty acid composition of germinated pumpkin seeds was very similar to that of the non-germinated sample. Generally, more significant changes in the fatty acid content are noted at longer germination times (Ahmed et al., 2024).

According to Table 4, ultrasonic treatment during hydration, irrespective of temperature and germination, did not result in substantial changes in mineral composition, even though some variations were statistically significant (<5%). However, while the treatments did not promote quantitative changes in mineral content, the action of endogenous enzymes synthesized during germination is expected to enhance the bioavailability of these nutrients. This enhancement occurs due to the hydrolysis of macronutrients, where minerals are typically complexed, as well as the reduction of antinutritional compounds within the matrix (Wu et al., 2023; Gandhi et al., 2022). Furthermore, pumpkin seeds are notable for their high content of iron (118.13 mg/100g in DM), zinc (70.15 mg/100g in DM), and manganese (54.40 mg/100g in DM) (Table 4), which exceed those found in other seeds (both germinated and non-germinated) reported in the literature, such as sunflower seeds (not detected, 5.10, and 5.36 mg/100g in DM, respectively) (Andressa et a., 2024), green beans (6.17, 3.11, and 0.95 mg/100g in DM, respectively) (Alkaltham et al., 2020), and green perilla (6.6, 0.99, and 3.81 µg/100g in DM, respectively) (Bhaswant et al., 2024). It is important to note that extrinsic factors such as soil type, light exposure, harvesting season, and irrigation significantly influence the mineral content of each seed type (Ghafoor et al., 2022). Therefore, further studies are necessary to assess the bioavailability of these nutrients.

Table 3. Impact of ultrasound assisted hydration at 25 °C and 45 °C on the fatty acid composition of germinated Pumpkin seed flour

Fatty acid (%)	Sample				
	non-germinated	**no-US 25 °C**	**US - 20 min 25 °C**	**no-US 45 °C**	**US - 20 min 45 °C**
C8:0 - Caprylic Acid	0.02 ± 0.00	N.d	N.d	N.d	N.d
C10:0 - Capric Acid	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	N.d	N.d
C12:0 - Lauric Acid	0.33 ± 0.03^{a}	0.10 ± 0.00^{b}	0.08 ± 0.00^{b}	0.06 ± 0.01^{b}	0.09 ± 0.01^{b}
C14:0 - Myristic Acid	0.25 ± 0.01^{a}	0.14 ± 0.00^{b}	0.12 ± 0.01^{b}	0.13 ± 0.00^{b}	0.14 ± 0.01^{b}
C16:0 - Palmitic Acid	17.66 ± 0.19^{a}	17.52 ± 0.06^{a}	17.65 ± 0.28^{a}	17.28 ± 0.23^{a}	17.43 ± 0.39^{a}
C16:1 - Palmitoleic Acid	0.09 ± 0.00^{a}	0.09 ± 0.01^{ab}	0.08 ± 0.00^{b}	0.08 ± 0.00^{b}	0.08 ± 0.00^{ab}
C17:0 - Margaric Acid	0.12 ± 0.00^{a}	0.13 ± 0.00^{a}	0.12 ± 0.00^{a}	0.13 ± 0.00^{a}	0.12 ± 0.00^{a}
C18:0 - Stearic Acid	9.41 ± 0.18^{a}	9.44 ± 0.47^{a}	9.57 ± 0.19^{a}	9.62 ± 0.59^{a}	9.55 ± 0.65^{a}
C18:1n9c - Oleic Acid	21.07 ± 0.12^{a}	21.09 ± 0.22^{a}	20.75 ± 0.09^{a}	21.25 ± 0.49^{a}	20.94 ± 0.02^{a}
C18:2n6c - Linoleic Acid	49.85 ± 0.38^{a}	49.64 ± 0.76^{a}	49.81 ± 0.02^{a}	49.60 ± 0.94^{a}	49.86 ± 0.24^{a}
C18:3n3 - α-linolenic Acid	0.28 ± 0.01^{c}	0.92 ± 0.05^{ab}	0.86 ± 0.01^{b}	0.94 ± 0.03^{a}	0.92 ± 0.00^{ab}
C20:0 - Arachidic Acid	0.48 ± 0.02^{b}	0.50 ± 0.04^{ab}	0.52 ± 0.02^{ab}	0.58 ± 0.03^{a}	0.46 ± 0.04^{b}
C20:1n9 - cis-11-eicosenoic Acid	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	0.11 ± 0.01^{a}	0.11 ± 0.00^{a}
C22:0 - Behenic Acid	0.18 ± 0.01^{a}	0.18 ± 0.01^{a}	0.19 ± 0.01^{a}	0.17 ± 0.02^{a}	0.20 ± 0.02^{a}
C20:5n3 - Eicosapentaenoic Acid	0.11 ± 0.01^{ab}	0.12 ± 0.01^{a}	0.09 ± 0.00^{b}	0.06 ± 0.01^{c}	0.09 ± 0.01^{b}

Different lowercase letters (column) indicate significant differences between different treatments by Tukey's test at 5% probability ($p<0.05$).

Table 4. Impact of ultrasound-assisted hydration at 25 °C and 45 °C on mineral profile of germinated Pumpkin seed flour

Mineral (mg/100g in DM)	**Sample**				
	non-germinated	**no-US 25 °C**	**US - 20 min 25 °C**	**no-US 45 °C**	**US - 20 min 45 °C**
Phosphorus (P)	0.73 ± 0.01[c]	0.78 ± 0.02[b]	0.83 ± 0.02[a]	0.82 ± 0.01[a]	0.80 ± 0.01[ab]
Potassium (K)	0.50 ± 0.01[c]	0.50 ± 0.01[cb]	0.51 ± 0.02[abc]	0.53 ± 0.01[ab]	0.54 ± 0.02[a]
Calcium (Ca)	0.05 ± 0.00[b]	0.06 ± 0.00[ab]	0.06 ± 0.00[a]	0.06 ± 0.00[a]	0.06 ± 0.00[ab]
Magnesium (Mg)	0.38 ± 0.00[c]	0.43 ± 0.00[b]	0.45 ± 0.01[a]	0.46 ± 0.01[a]	0.46 ± 0.01[a]
Copper (Cu)	12.21 ± 0.71[a]	10.90 ± 0.44[a]	11.57 ± 0.49[a]	12.13 ± 0.55[a]	11.57 ± 2.13[a]
Iron (Fe)	118.13 ± 5.84[a]	102.36 ± 3.52[b]	112.56 ± 6.52[ab]	118.21 ± 3.22[a]	115.76 ± 1.52[a]
Zinc (Zn)	70.15 ± 1.19[b]	78.02 ± 2.77[ab]	81.82 ± 4.16[a]	85.23 ± 4.13[a]	85.68 ± 1.52[a]
Manganese (Mn)	54.40 ± 0.70[b]	57.81 ± 0.66[b]	62.43 ± 1.10[a]	63.57 ± 2.09[a]	63.77 ± 1.71[a]
Sodium (Na)	0.01 ± 0.00[c]	0.05 ± 0.00[a]	0.05 ± 0.00[a]	0.04 ± 0.00[b]	0.05 ± 0.00[a]

Different lowercase letters (column) indicate significant differences between different treatments by Tukey's test at 5% probability ($p<0.05$). DM: dry matter.

3.2.2. Gamma-aminobutyric acid (GABA) and *in vitro* antioxidant activity

An important substance accumulated during germination is GABA, a non-protein amino acid primarily produced through the irreversible α-decarboxylation of L-glutamic acid, catalyzed by glutamic acid decarboxylase (GAD) (Deore et al., 2023; Yu et al., 2024). GABA is recognized for its diverse physiological functions, making it a valuable ingredient in functional foods and nutraceuticals (Sun et al., 2022). According to the FDA (2015), the estimated daily intake of GABA from dietary sources in the United States is approximately 418 mg per person, with variations across age groups.

As shown in Figure 6A, GABA content after germination was significantly influenced by hydration temperature and sonication. GABA levels increased by 239% in the non-sonicated samples and by 285% in the sonicated samples after hydration at 45 °C followed by germination. The hydration and germination can induce stress conditions in seeds, activating endogenous enzymes, particularly glutamate decarboxylase, and thus increasing GABA concentration (Li et al., 2022). Additionally, ultrasound exposure enhances energy metabolism during seed germination, further promoting GABA accumulation by increasing glutamate decarboxylase activity and supplying more L-glutamate (Wu et al., 2023; Naumenko & Kalininina, 2022). The literature indicates a significant increase in GABA content with ultrasound application during germination. For example, Ding et al. (2018) reported that wheat flour with an initial GABA content of 14.68 mg/100g increased to 49.72 mg/100g after germination and further to 64.98 mg/100g with sonication.

GABA is naturally present in various seeds, including beans, rice, and sesame (Vann et al., 2020). Wu et al. (2023) monitored GABA content over 96 hours of germination, noting that the control sample's initial concentration of 4.16 mg/100g rose by 41.3% to 5.88 mg/100g by the end of the period. Similarly, Tiansawang et al. (2016) observed a significant increase in GABA content in bean samples, from 13.25 mg/100g to 80.68 mg/100g after 48 hours of germination. In comparison, pumpkin seeds exhibited substantial GABA concentrations that were further enhanced by ultrasound and elevated temperature.

Recently, there has been growing interest in functional foods rich in GABA, highlighting the potential of seeds as bioactive food ingredients. These results suggest promising opportunities for producing GABA-enriched baked goods (Cornejo et al., 2015a). Jabeen et al. (2024) developed a functional beverage based on germinated brown rice, initially containing 42.12 mg/100g of GABA. However, this content decreased significantly during storage, dropping to 32.43 mg/100g at 25 °C and 35.32 mg/100g at 4 °C after six months. These findings indicate that while it is feasible to create GABA-rich beverages, low-temperature storage can help minimize GABA loss over time (Jabeen et al., 2024).

Germination is a technological process aimed at increasing the availability of antioxidants through biochemical transformation (Ti et al., 2014). In this study, germination significantly enhanced the antioxidant activity against both ABTS and DPPH radicals (Figures 6B and 6C) ($p<0.05$). Specifically, the antioxidant capacity for the ABTS radical in samples sonicated during hydration at 25 °C increased by up to 172%, while the DPPH radical showed an increase of up to 74% compared to non-germinated samples. However, when the hydration temperature was raised from 25 °C to 45 °C, these increases were less pronounced, reaching only 132% for ABTS and 44% for DPPH. The enhancement in antioxidant activity promoted by ultrasound can be attributed to ultrasonic cavitation, which facilitates the decomplexation of antioxidant compounds during germination, including phenolic compounds, GABA, chlorophyll, carotenoids, and glucosinolates (Yu et al., 2016; Zhang et al., 2020). Conversely, the increase in temperature minimized the rise in antioxidant activity in germinated samples, possibly due to the degradation of sensitive antioxidant compounds such as flavonoids and phenolic acids (Song et al., 2020).

Based on the results obtained, the antioxidant activity of pumpkin seed flour subjected to germination and ultrasound treatment demonstrated superior values compared to other germinated raw materials. For instance, soybean exhibited an antioxidant activity of 13.0 μmol of Trolox equivalents/100g after 5 days of germination (Huang et al., 2014), while mung beans reached 20.0 μmol of Trolox equivalents/100g under similar conditions (Huang et al., 2014). Additionally, rice attained 100.09 μmol of Trolox equivalents/100g after 72 hours of germination (Wu et al., 2022). This superior performance underscores the potential of germinated and sonicated pumpkin seed flour as a promising source of antioxidant compounds.

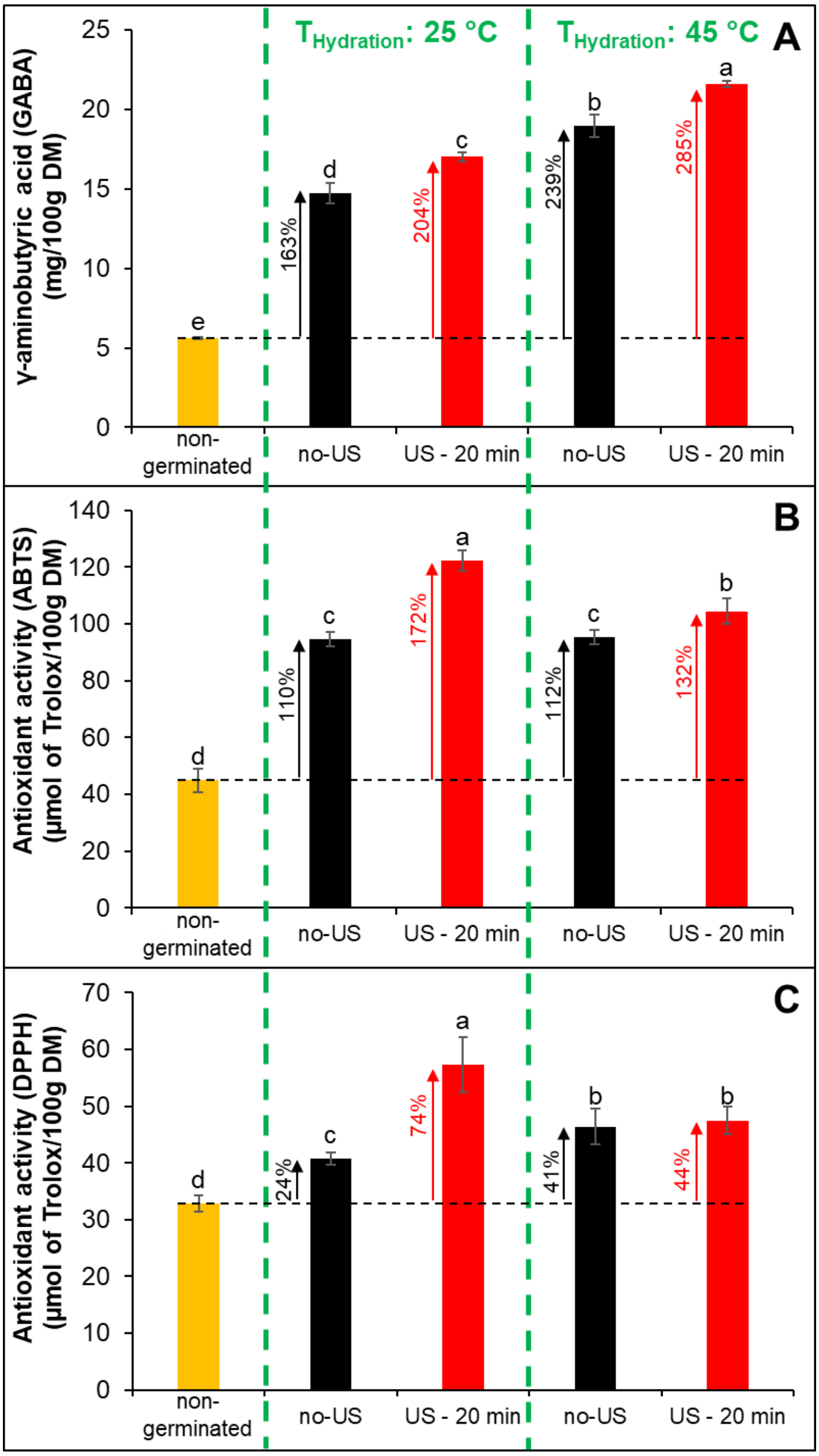

Figure 6. Impact of ultrasound-assisted hydration at 25°C and 45°C on GABA content (A), and antioxidant activity, measured by ABTS (B) and DPPH (C) radicals, of germinated pumpkin seed flours. Different lowercase letters indicate significant differences between different treatments by the 5% Tukey test ($p<0.05$).

3.2.3. Color parameters

Color is a critical quality attribute in foods and can be influenced by processing (Xia & Li, 2018). The color coordinates (L*, a*, and b*) of the pumpkin seed flour exhibited significant changes after germination (Figures 7A, 7B, 7C). Specifically, luminosity (L*) increased from 60.3 to 64.8, while redness (positive a*) decreased from 4.4 to 2.9, and yellowness (positive b*) decreased from 29.4 to 27.6. The increase in luminosity corresponds to a visually lighter color (Figure 7E). Similar results were reported by Sofi et al. (2023) for germinated chickpea flour samples. Although statistical differences were noted for the a* parameter, these differences are minimal in practical terms. Moreover, the application of ultrasonic pretreatment during hydration and the increase in hydration temperature did not significantly affect luminosity or the b* parameter ($p>0.05$).

The observed change in luminosity can be attributed to leaching, which involves the transfer of colored components, such as carotenoids (especially β-carotene), into the aqueous environment during hydration, contributing to the yellow hue of pumpkin seeds (Oli et al., 2016; Shen et al., 2020).

The total color difference (ΔE) quantifies the extent of color change in the flours after treatments. According to Xiong et al. (2023), ΔE values less than 1 indicate no discernible difference, values between 1 and 3 indicate small differences, and values above 3 indicate noticeable differences compared to the control. In this study, the ΔE values of the germinated samples, with or without US intervention, exceeded 3 when compared to the non-germinated sample, suggesting that the color differences are easily perceptible to the naked eye, as illustrated in Figure 7E. However, the ΔE values between the germinated samples, with or without US at the same temperature, did not differ significantly ($p>0.05$), indicating that sonication during hydration did not alter the coloration between the germinated samples.

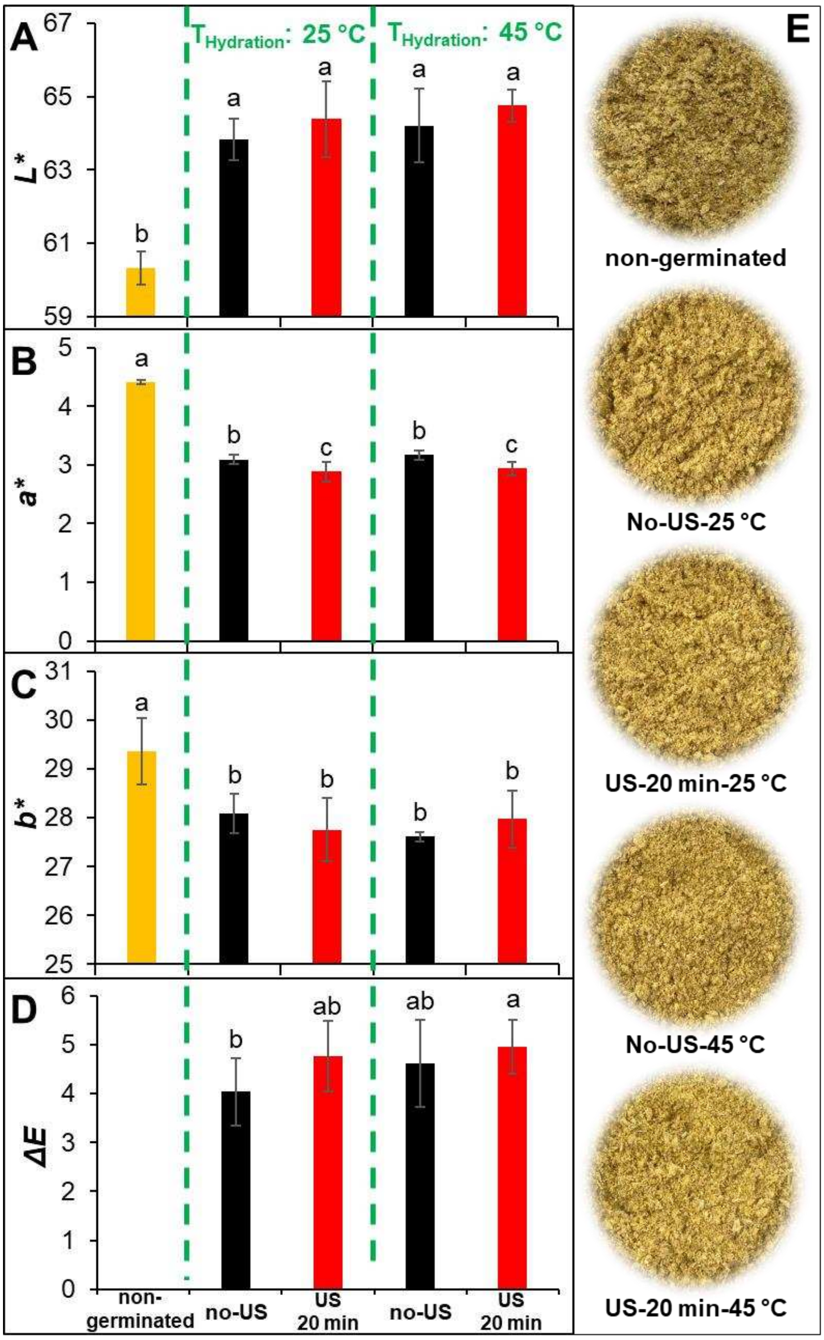

Figure 7. Impact of ultrasound-assisted hydration at 25°C and 45°C on color parameters of germinated pumpkin seed flours. Different lowercase letters indicate significant differences between different treatments by the 5% Tukey test ($p<0.05$).

3.3. Techno-functional properties

Figure 8 shows the techno-functional properties of the flours. The non-germinated flour exhibited values of WHC, OHC, swelling index, solubility index, FC, and FS in the ranges of 1.47 (g/g), 0.92 (g/g), 3.89 (g/g), 7.79 (g/100g), 18.7%, and

21.7%, respectively. All these evaluated properties improved with germination by up to 39%, 27%, 8%, 40%, 57%, and 124%, respectively ($p<0.05$). The increase in these properties may be attributed to the partial hydrolysis of polysaccharides and proteins by metabolic enzymes, as well as structural changes (evidenced by intrinsic fluorescence and FTIR), promoting the formation of polar groups in low molecular weight proteins (Handa et al., 2017).

A similar pattern of improvement in functional properties due to germination has been observed in various studies. Many researchers report an increase in WHC with germination. For instance, Sofi et al. (2023) found that chickpea flour increased WHC from 1.7 g/g in non-germinated samples to 2.2 g/g in germinated samples. Similarly, Liu et al. (2018) reported an increase in WHC of bean flour from 2.3 g/g in non-germinated samples to 3.1 g/g after 72 hours of germination. In the case of wheat flour, which is widely used in baking, the WHC values reported in the literature range from 2.1 g/g in non-germinated flour to 2.8 g/g in germinated flour (Dhillon et al., 2020). Other studies have documented increased OHC with germination. For example, Kristanti et al. (2023) observed an increase in OHC from 1.93 g/g to 2.16 g/g in sorghum flour, while Romano et al. (2023) reported an increase from 1.39 g/g to 2.49 g/g in lentil flour. Similarly, Mao et al. (2024) noted a rise in OHC from 0.70 g/g to 0.75 g/g in chickpea flour.

Moreover, germination not only enhances WHC and OHC but also improves other functional properties, such as swelling and solubility indices. Sofi et al. (2023) documented significant increases in these indices for germinated chickpea flour. The results showed that the swelling index increased from 7.4 g/g to 8.3 g/g, while the solubility index increased from 18.1 g/100g to 25.1 g/100g. Finally, Gnanwa et al. (2021) observed that germination positively impacted the foaming capacity and foam stability of germinated bean flour, with an increase in foaming capacity from 22.08% to 26.74% and foam stability from 19.23% to 21.75%.

A distinguishing feature of this study, which has yet to be reported in the literature, is the effect of ultrasound pretreatment during the hydration and germination of pumpkin seeds on the functional quality of the resulting flour. All previously mentioned functional properties, which showed improvements during germination, were further enhanced with sonication.

Regarding the ultrasound pretreatment and hydration conducted at different temperatures, an increase ($p < 0.05$) in WHC to 2.1 g/g was observed, representing a 49% increase for samples germinated with US compared to non-germinated samples, and a 10% increase compared to germinated samples without US at 25 °C (Figure 4A). In parallel, similar responses were obtained for WHC when the hydration temperature was increased to 45 °C. The enhanced water absorption capacity may be related to structural changes in macronutrients, such as starch, as demonstrated in previous microstructure analyses, which showed increased interaction sites with water (Dogruer et al., 2023).

In terms of oil-holding capacity, increasing the hydration temperature led to substantial increases of 51% and 59% in OHC for samples without ultrasound (1.39 g oil/g sample) and sonicated samples (1.47 g/g), respectively, compared to the non-germinated sample (0.92 g/g) (Figure 8B). Higher temperatures can cause the polypeptide chains to unfold, exposing hydrophilic groups and hydrophobic side chains (Yu et al., 2023).

Sonication was also effective in increasing the swelling and solubility indices. For the swelling index, increases of 8% and 17% were observed for non-sonicated and sonicated samples at 25 °C, respectively, and 12% and 29% for the same samples at 45 °C ($p < 0.05$). This indicates that higher hydration temperatures enhanced the swelling effect. The solubility index of the flour ranged from 7.79 g/100g for the non-germinated sample to 13.10 g/100g for the sonicated and germinated sample at 45 °C, with higher values observed for samples treated with ultrasound. These US-treated samples exhibited increases of up to 62% (at 25 °C) and 68% (at 45 °C) compared to the non-germinated sample. These results may be related to the physical damage incurred by the treated seeds, attributed to modifications in the structure of the starch granules (as observed in sections 3.1.3 and 3.2.4) as well as the dissociation of proteins (Amini et al., 2015; Kaur & Gill, 2019).

For foaming capacity, maximum increases of 114% and 143% were observed for the sonicated sample during hydration at 25 °C and 45 °C, respectively, compared to the non-germinated sample. Regarding foam stability, increases of 208% and 173% were noted for the sonicated sample during hydration at 25 °C and 45 °C, respectively, compared to the non-germinated sample. FC is influenced by the transport, penetration, and reorganization of molecules at the air/water interface. The cavitation phenomenon likely resulted in the exposure of hydrophobic groups, contributing to enhanced interaction at the air-water interface. Furthermore, it led to a partially unfolded protein structure and improved foaming properties (Gani et al., 2022).

Considering the results of the properties presented and aiming for the application of this flour in the food sector, although ultrasound improved WHC and OHC properties, more significant results were observed for the solubility index, FC, and FS, demonstrating that this flour can be particularly beneficial for gluten-free products (Singh et al., 2017).

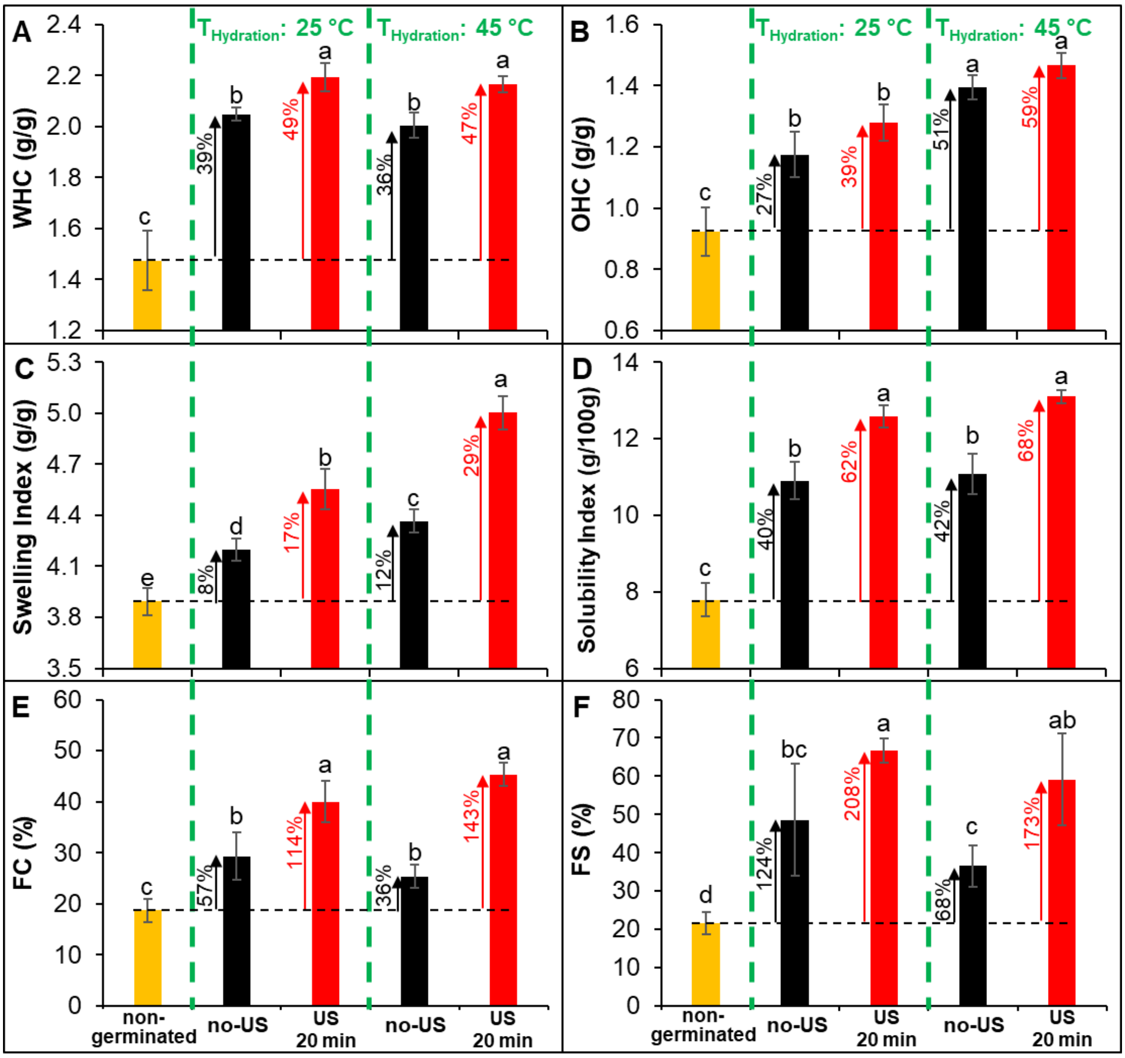

Figure 8. Impact of ultrasound assisted hydration at 25°C and 45°C on water holding capacity-WHC (A), oil holding capacity-OHC (B), swelling index (C), solubility index (D), foam capacity-FC (E) and foam stability-FS (F) in germinated pumpkin seed flour. The concentration of WHC and OHC was expressed as g of water/oil per g of dry

matter (g/g). Foaming and foaming stability in terms of percentage. Different lowercase letters indicate significant differences between different treatments by the 5% Tukey test ($p<0.05$)

3.4. General discussion, possible applications and importance of the study

The growing demand for healthier, sustainable, and more natural plant-based foods has led to increased interest in germinated flour due to its favorable nutritional profile and sensory qualities (Lan et al., 2023). This study aimed to utilize pumpkin seeds, which are often viewed as waste or by-products, to produce flours with enhanced quality. This approach not only reduces the negative environmental impact associated with underutilized vegetable food sources (Miyahira et al., 2021) but also valorizes agro-industrial by-products, thereby contributing to sustainable food development (Lan et al., 2023).

As discussed in the previous sections, the application of US-assisted hydration and germination provided various nutritional and techno-functional advantages, thereby improving the flour's quality and its potential for diverse food applications. The flours produced in this study can be utilized in a wide range of food products, including breads, cakes, cookies, and beverages, particularly for vegetarian and vegan consumers (Jambrak et al., 2014). The enhancements in nutritional properties, such as increased GABA content and antioxidant activity, make these flours suitable for developing functional foods tailored to health-conscious consumers (Hou et al., 2023). For instance, a previous study demonstrated a significant increase in GABA levels in germinated red rice yogurt, with values rising from 2.05 mg/100 g to 21.32 mg/100 g after 32 h of germination. In this study, the GABA content in the flour increased from 5.6 mg/100 g to 21.6 mg/100 g following US-assisted hydration and 72 h of germination. This positions the flour as a promising ingredient for developing fermented beverages analogous to dairy products, with the potential to enhance GABA levels in these formulations.

Moreover, the improved techno-functional properties of the flour suggest that it can partially substitute other flours in food formulations, enhancing the texture and stability of the final products (Angelis et al., 2024). Saeed et al. (2023) demonstrated that combining germinated chickpea flour with wheat flour resulted in cookies with enhanced functional properties, including improved water and oil holding capacities. The incorporation of germinated chickpea flour increased antioxidant activity, flavonoid, and phenolic compound content, along with favorable textural and sensory characteristics. Additionally, Bhinder et al. (2022) showed that germinating tartary buckwheat flour for 48 h improved its functionality, with notable increases in water holding capacity and foaming capacity, leading to muffins with enhanced rheological behavior and reduced glycemic index. Similarly, the results from this study indicated that pumpkin seed flour, after US-assisted hydration and germination, exhibited improvements in water holding capacity (up to 2.16 g/g), oil holding capacity (up to 1.47 g/g), and foaming capacity (up to 143%), making it a viable candidate for the production of functional cookies and muffins.

Transforming pumpkin seeds, often regarded as waste, into high-value functional ingredients represents a significant opportunity for the food sector. Furthermore, innovative techniques like ultrasound treatment provide a sustainable method for enhancing food ingredient quality, contributing to a circular economy and minimizing food waste. The findings from this study can stimulate the large-scale utilization of sprouted flours, promoting product diversification within the healthy and sustainable food market.

While germination has shown effectiveness in enhancing nutritional and technological characteristics, ultrasound treatment at elevated temperatures yielded even more substantial improvements. Nonetheless, in terms of industrial application, ultrasound-assisted hydration using two sonication cycles at 25 °C proved to be the most practical approach, offering significant benefits while minimizing energy consumption and facilitating large-scale implementation.

Finally, it is important to note that there remains a scarcity of research investigating the performance of these flours in actual food products, particularly as substitutes for wheat flour in baking applications with ultrasound pretreatment. Therefore, further studies are warranted to explore the potential of these flours in healthy food development and to assess the effectiveness of their functional components during processing and consumption. Future research efforts should focus on applying germinated pumpkin seeds to create new products and expand their application in the food industry.

4. Conclusion

This study demonstrated that germination, enhanced by ultrasound technology and increased temperature during pumpkin seed hydration, positively impacted the quality of the resulting flour. In addition to maintaining the mineral and fatty acid profiles, the impact of ultrasound-assisted hydration and germination on the structural components of pumpkin seeds resulted in improvements in physicochemical, bioactive, and techno-functional properties. Therefore, the application of ultrasound technology during seed hydration, in conjunction with germination, is an effective method for transforming pumpkin seeds into functional flour with significant potential as a valuable food source.

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CONCLUSÕES GERAIS

As sementes de abóbora, embora tradicionalmente consideradas resíduos agroindustriais, são ricas em nutrientes e possuem propriedades nutracêuticas valiosas, tornando-se potenciais aditivos alimentares na indústria de alimentos. Este estudo, pioneiro em sua abordagem, demonstrou que a aplicação de ultrassom em pulsos durante a fase de hidratação das sementes de abóbora pode melhorar significativamente a cinética de hidratação, a taxa de germinação e a qualidade das farinhas obtidas. Os resultados indicaram um aumento nos teores de proteína e lipídios nas amostras tratadas com ultrassom em comparação com as amostras germinadas sem ultrassom e as não germinadas. Além disso, o tratamento ultrassônico durante a hidratação promoveu um aumento nos níveis de açúcares redutores, compostos fenólicos totais e proteínas solúveis, destacando seus benefícios à saúde em relação à amostra germinada sem intervenção ultrassônica.

O estudo também evidenciou que a germinação, aprimorada pela tecnologia de ultrassom e pelo aumento da temperatura durante a hidratação das sementes, impactou positivamente a qualidade da farinha resultante. Esse processo manteve os perfis de minerais e ácidos graxos, ao mesmo tempo que melhorou as propriedades físico-químicas, bioativas e tecno-funcionais das sementes de abóbora. Dessa forma, a aplicação da tecnologia de ultrassom em conjunto com a germinação se mostrou uma estratégia eficaz para transformar sementes de abóbora em uma farinha funcional, com um potencial significativo como fonte alimentar de alto valor.