



**HAL**  
open science

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

Flaviana Coelho Pacheco, Jeferson Silva Cunha, Irene Andressa, Fábio Ribeiro dos Santos, Ana Flávia Coelho Pacheco, Gabriela Aparecida Nalon, Paulo Henrique Costa Paiva, Alline Artigiani Lima Tribst, Pedro Augusto, Bruno Ricardo de Castro Leite Junior

### ► To cite this version:

Flaviana Coelho Pacheco, Jeferson Silva Cunha, Irene Andressa, Fábio Ribeiro dos Santos, Ana Flávia Coelho Pacheco, et al.. Ultrasound-assisted intermittent hydration of pumpkin seeds: Improving the water uptake, germination and quality of a clean label ingredient. *Food and Bioprocess Technology*, 2024, 10.1007/s11947-024-03487-w . hal-04625787

**HAL Id: hal-04625787**

**<https://hal.science/hal-04625787v1>**

Submitted on 26 Jun 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Pacheco, F.C., Cunha, J.S., Andressa, I. Santos, F. R., Pacheco, A.F.C.; Nalon, G.A., Paiva, P.H.C., Tribst, A.A.L., Augusto, P.E.D., Leite Júnior, B.R.C. Ultrasound-Assisted Intermittent Hydration of Pumpkin Seeds: Improving the Water Uptake, Germination, and Quality of a Clean Label Ingredient. *Food and Bioprocess Technology* (2024). <https://doi.org/10.1007/s11947-024-03487-w>.

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

Flaviana Coelho Pacheco<sup>1</sup>, Jeferson Silva Cunha<sup>1</sup>, Irene Andressa<sup>1</sup>, Fábio Ribeiro dos Santos<sup>1</sup>, Ana Flávia Coelho Pacheco<sup>2</sup>, Gabriela Aparecida Nalon<sup>1</sup>, Paulo Henrique Costa Paiva<sup>2</sup>, Alline Artigiani Lima Tribst<sup>3</sup>, Pedro Esteves Duarte Augusto<sup>4</sup>, Bruno Ricardo de Castro Leite Júnior<sup>1\*</sup>

<sup>1</sup> Department of Food Technology, Federal University of Viçosa, Avenue Peter Henry Rolfs, s/n, University Campus, 36570-900, Viçosa, Brazil

<sup>2</sup> Cândido Tostes Dairy Institute, Agricultural Company of Minas Gerais (EPAMIG), Tenente Luiz de Freitas, 116, 36045-560, Juiz de Fora, Brazil

<sup>3</sup> Center for Food Studies and Research (NEPA). Coordination of Interdisciplinary Research Centers and Nuclei (COCEN). State University of Campinas (UNICAMP), Albert Einstein, 291, 13083-852, Campinas, Brazil.

<sup>4</sup> Université Paris-Saclay, CentraleSupélec, Laboratoire de Génie des Procédés et Matériaux, Centre Européen de Biotechnologie et Bioéconomie (CEBB), 3 rue des Rouges Terres 51110, Pomacle, France.

\* Corresponding author.

**Address** Avenue Peter Henry Rolfs, s/n, University Campus, 36570-900, Viçosa, Brazil. **E-mail** bruno.leitejr@ufv.br (Bruno R. de C. Leite Júnior)

## **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 ( $\leq 54.3\%$ ), decreased the germination lag phase ( $\leq 19.3\%$ ), and increased maximum germination capacity ( $\leq 5\%$ ) ( $p < 0.05$ ). Furthermore, ultrasonic treatment led to greater rootlet length ( $\leq 19.4\%$ ) after germination. Regarding the flour quality, ultrasound increased protein and lipid content ( $\leq 23\%$  and  $\leq 8.4\%$ , 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 ( $\leq 79\%$ ), phenolic compounds ( $\leq 81\%$ ), and soluble protein ( $\leq 19.8\%$ ) 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.

## **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 accelerating 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.

## **Materials and methods**

## **Raw material and general description of the process**

Pumpkin seeds (*Cucurbita moschata* D.) were supplied by the company Doces Mirahy (Miraf, 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.

### **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<sup>-1</sup> 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).

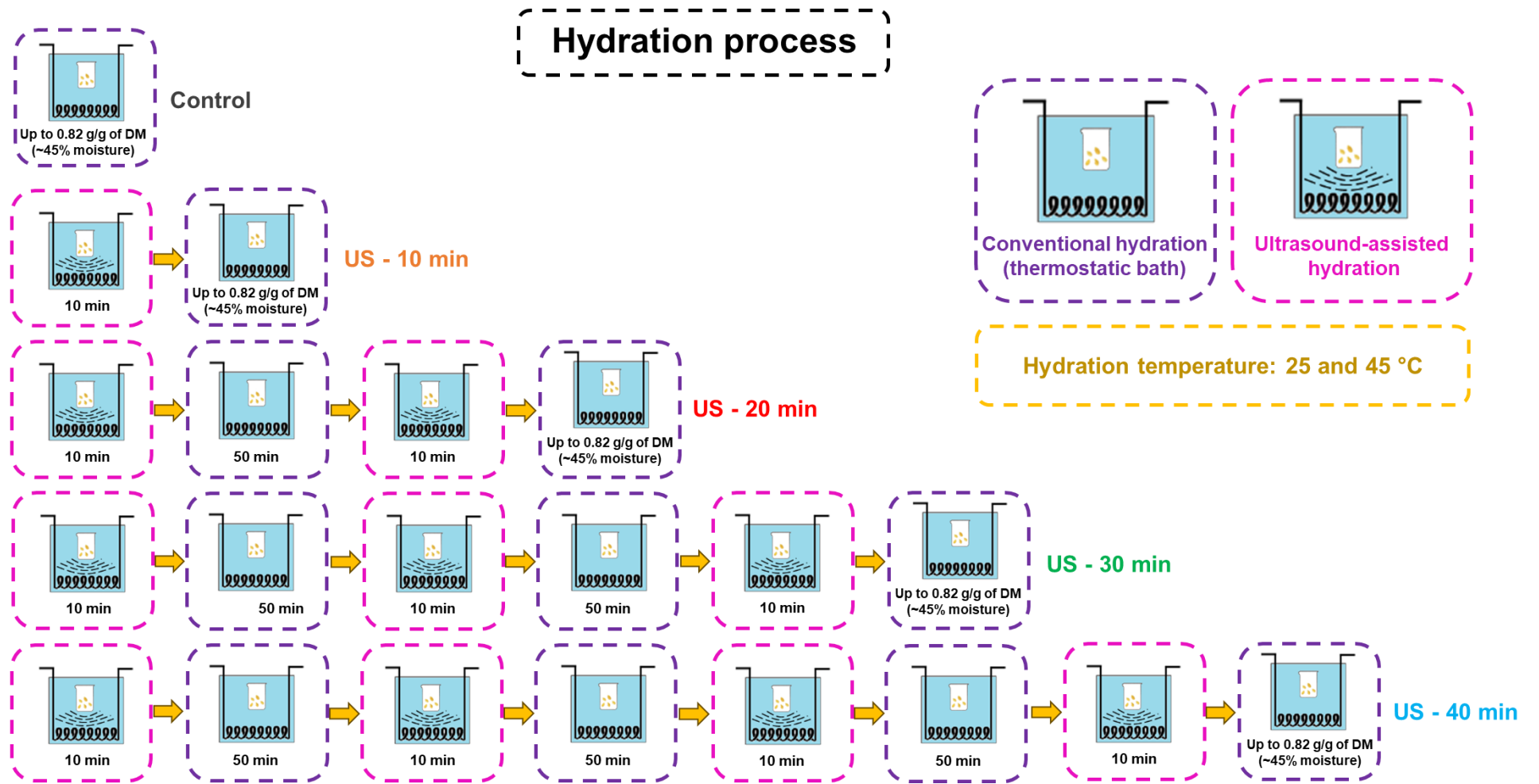


Fig. 1 Flow chart of the work

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  $25 \pm 2$  °C (optimum temperature for pumpkin seeds germination, according to Zehtab-Salmasi (2006) and  $45 \pm 2$  °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).

### **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.

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

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.

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

Where  $\alpha$  is the shape parameter (dimensionless), which is also associated with the initial rate of water absorption, and  $\beta$  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 ( $t \rightarrow \infty$ ) (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 ( $R_{adj}^2$ ) (Equation 4 (4)) and root-mean-square error (RMSE) (Equation 5 (5)) (Polachini et al., 2023).

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

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

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

## **Evaluation of the germination process of pumpkin seeds previously subjected to the US-assisted hydration process**

### **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<sup>2</sup>) 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):

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

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:  $\lambda$  (h), which represents the germination lag phase (time needed to start germination);  $k_g$  ( $\text{h}^{-1}$ ), which means the germination rate; and  $G_{max}$  (%), which indicates the maximum percentage of germination.

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

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 ( $R^2$ ).

### **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.

### **Composition of pumpkin seed flours**

#### **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.

### **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 °C 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 °C 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).

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

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

### **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).

### **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.

## **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).

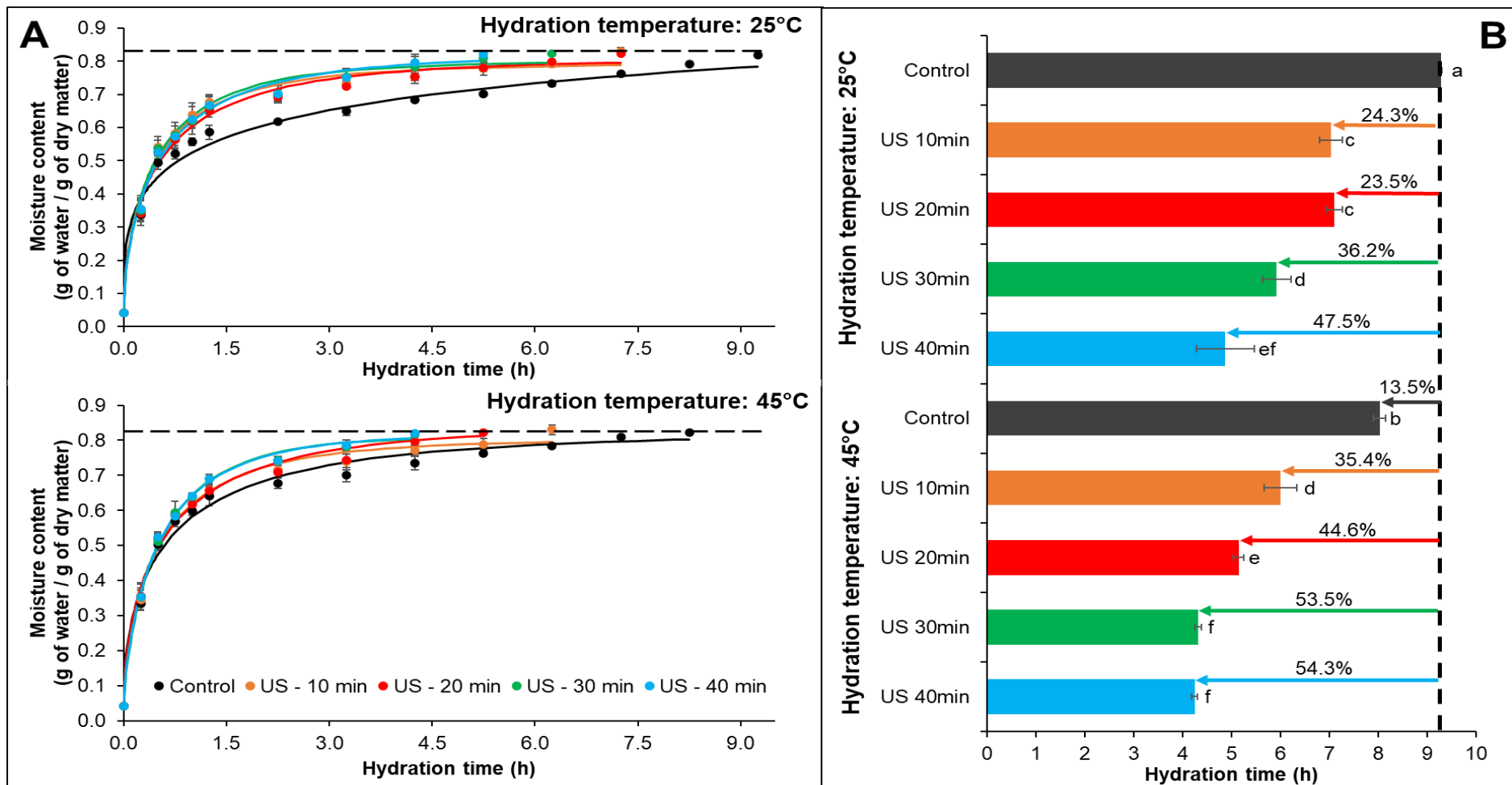
## **Experimental design and statistical analysis**

The processes were performed in three replicates, and each analysis was performed in triplicate. The results were expressed as mean  $\pm$  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).

## **Results and Discussion**

### **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  $t \rightarrow 0$ . 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

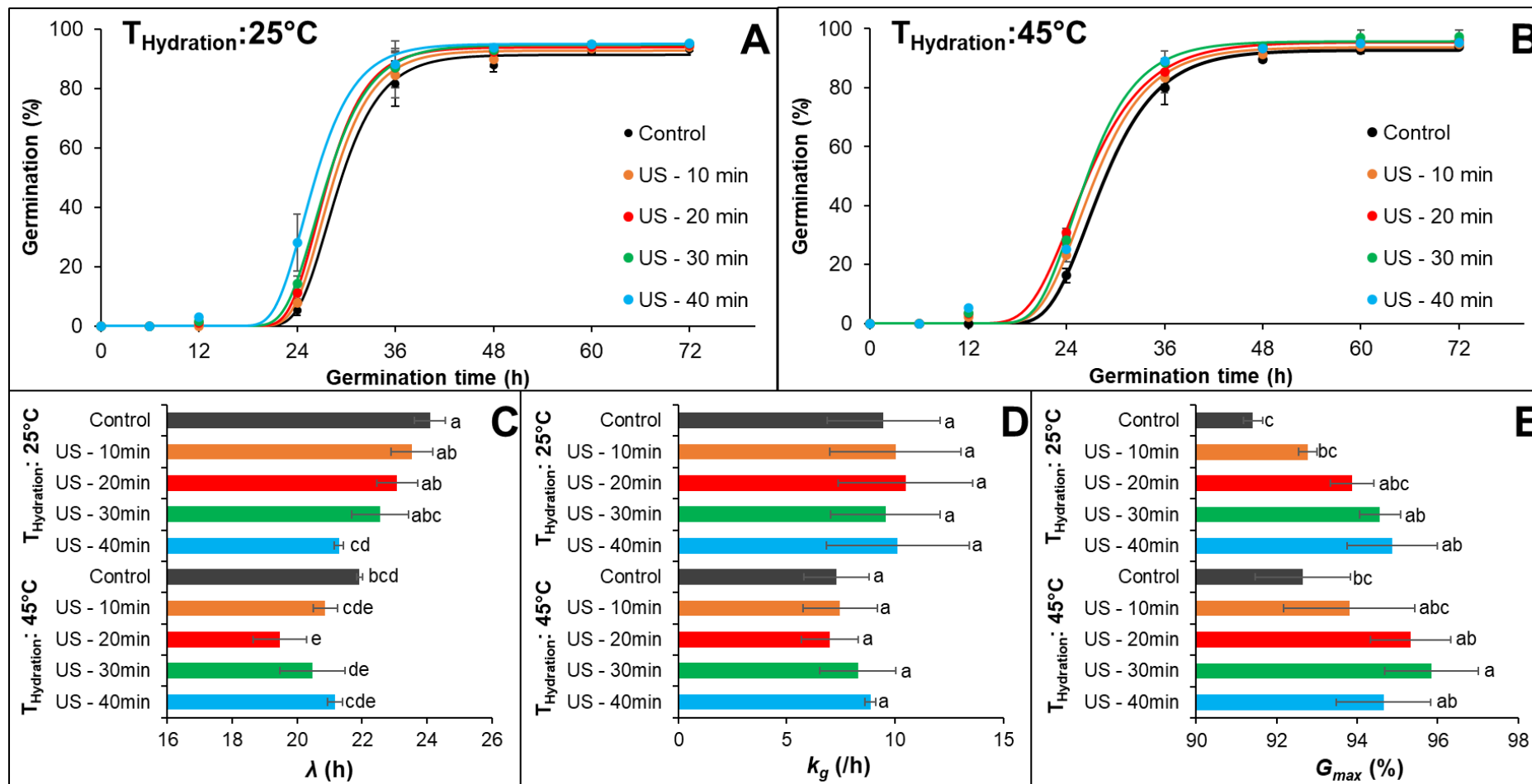
<b>Peleg Model</b>						
<b>Sample</b>	<b>Hydration temperature</b>	$k_1$ (g of dry matter h g <sup>-1</sup> ·of water)	$k_2$ (g of dry matter g <sup>-1</sup> of water)	$X_{eq}$ (g of water g <sup>-1</sup> of dry matter)	$R^2_{adj}$	$RMSE$
Control	25 °C	0.55 ± 0.05 <sup>a</sup>	1.35 ± 0.00 <sup>a</sup>	0.78 ± 0.00 <sup>c</sup>	>0.979	<0.0352
US 10min		0.43 ± 0.06 <sup>a</sup>	1.27 ± 0.01 <sup>b</sup>	0.83 ± 0.01 <sup>bc</sup>	>0.992	<0.0211
US 20min		0.48 ± 0.06 <sup>a</sup>	1.27 ± 0.01 <sup>bc</sup>	0.83 ± 0.01 <sup>abc</sup>	>0.993	<0.0200
US 30min		0.47 ± 0.10 <sup>a</sup>	1.24 ± 0.02 <sup>bc</sup>	0.85 ± 0.02 <sup>ab</sup>	>0.993	<0.0188
US 40min		0.47 ± 0.07 <sup>a</sup>	1.24 ± 0.04 <sup>bc</sup>	0.85 ± 0.03 <sup>ab</sup>	>0.992	<0.0185
Control	45 °C	0.50 ± 0.03 <sup>a</sup>	1.28 ± 0.02 <sup>ab</sup>	0.82 ± 0.01 <sup>bc</sup>	>0.990	<0.0219
US 10min		0.47 ± 0.05 <sup>a</sup>	1.25 ± 0.04 <sup>bc</sup>	0.84 ± 0.03 <sup>ab</sup>	>0.996	<0.0158
US 20min		0.47 ± 0.05 <sup>a</sup>	1.24 ± 0.05 <sup>bc</sup>	0.85 ± 0.03 <sup>ab</sup>	>0.990	<0.0208
US 30min		0.48 ± 0.01 <sup>a</sup>	1.19 ± 0.02 <sup>c</sup>	0.88 ± 0.01 <sup>a</sup>	>0.991	<0.0197
US 40min		0.48 ± 0.02 <sup>a</sup>	1.19 ± 0.02 <sup>c</sup>	0.88 ± 0.02 <sup>a</sup>	>0.995	<0.0152
<b>Weibull Model</b>						
<b>Sample</b>	<b>Hydration temperature</b>	$\alpha$ (dimensionless)	$\beta$ (h)	$R^2_{adj}$	$RMSE$	
Control	25 °C	0.33 ± 0.02 <sup>b</sup>	3.29 ± 1.63 <sup>a</sup>	>0.986	<0.0286	
US 10min		0.63 ± 0.01 <sup>a</sup>	0.52 ± 0.07 <sup>b</sup>	>0.989	<0.0256	
US 20min		0.59 ± 0.07 <sup>a</sup>	0.62 ± 0.06 <sup>b</sup>	>0.990	<0.0243	
US 30min		0.65 ± 0.09 <sup>a</sup>	0.52 ± 0.02 <sup>b</sup>	>0.992	<0.0328	
US 40min		0.62 ± 0.11 <sup>a</sup>	0.61 ± 0.07 <sup>b</sup>	>0.992	<0.0193	
Control	45 °C	0.54 ± 0.09 <sup>a</sup>	0.74 ± 0.17 <sup>b</sup>	>0.989	<0.0260	
US 10min		0.62 ± 0.06 <sup>a</sup>	0.58 ± 0.17 <sup>b</sup>	>0.993	<0.0201	
US 20min		0.60 ± 0.18 <sup>a</sup>	0.67 ± 0.10 <sup>b</sup>	>0.992	<0.0183	
US 30min		0.75 ± 0.13 <sup>a</sup>	0.57 ± 0.04 <sup>b</sup>	>0.992	<0.0193	
US 40min		0.73 ± 0.10 <sup>a</sup>	0.58 ± 0.03 <sup>b</sup>	>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  $\alpha$  and  $\beta$  parameters of the Weibull model. The parameter  $\alpha$  is related to the mechanisms of water migration from the surroundings towards the center of the seed. According to Corzo et al. (2010),  $\alpha$  values below 1 indicate that the external resistance to water transfer is much lower than the internal resistance for hydration processes. All  $\alpha$  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  $\alpha$  values ( $\leq 127\%$ ) 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 °C ( $p > 0.05$ ). On the other hand, although the  $\alpha$  values of the sonicated samples were higher, the kinetic parameter  $\beta$ , 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 °C (Fig. 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:  $\lambda$ , 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).  $\lambda$  (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  $\lambda$  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).

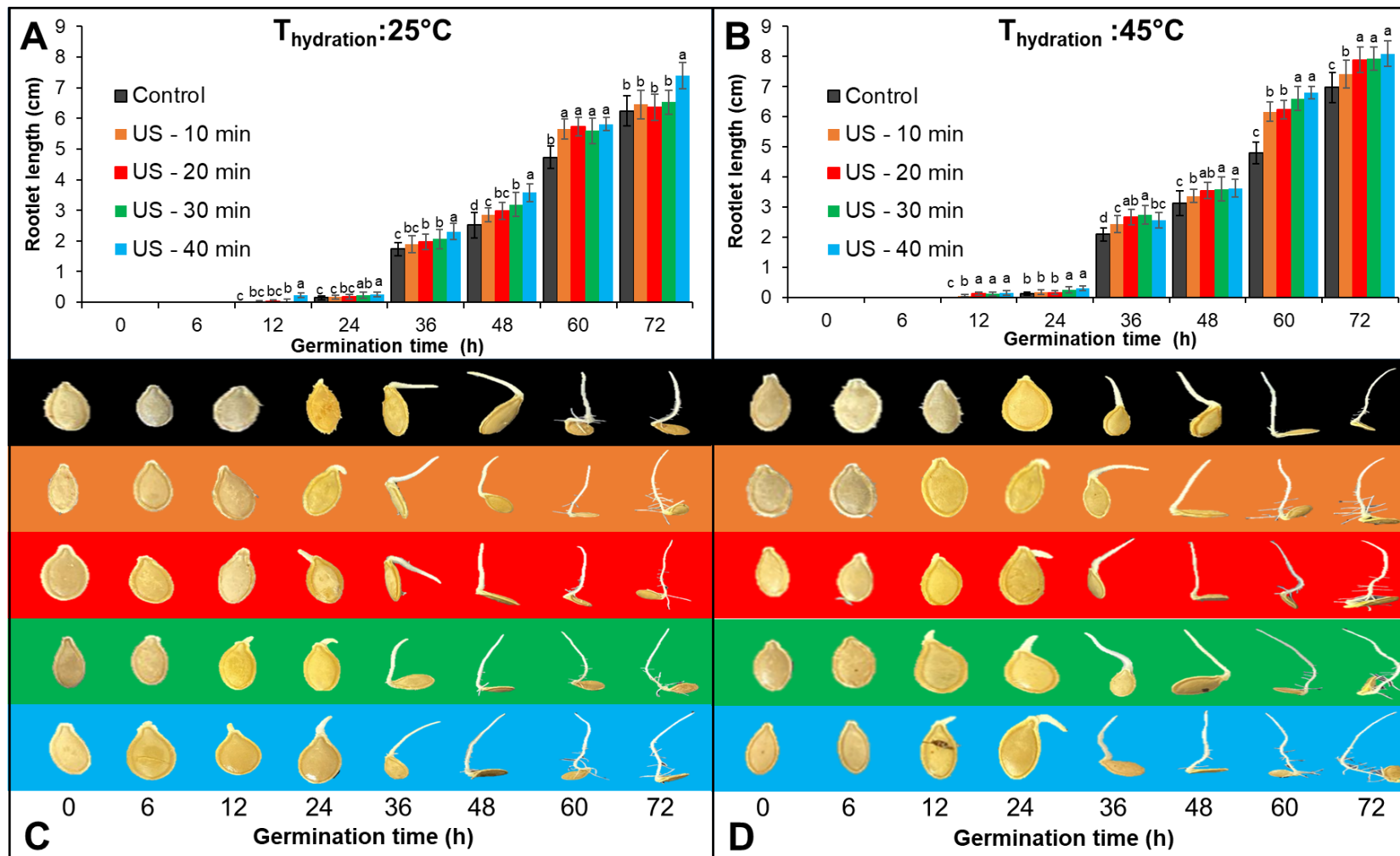
Based on these results, it was observed that samples hydrated under US, especially with 4 cycles of 10 min at 25 °C and 45 °C, showed a shorter AGT (reduction of up to 12.1% - Table 2). This result is justified by the decrease in the latency phase, which increased the percentage of germination, especially 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 (%)		
			1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day
Control	25 °C	1.57 ± 0.05 <sup>a</sup>	5.22 ± 1.70 <sup>e</sup>	87.66 ± 2.10 <sup>d</sup>	93.02 ± 0.53 <sup>c</sup>
US - 10min		1.55 ± 0.06 <sup>a</sup>	7.89 ± 2.22 <sup>de</sup>	89.84 ± 2.59 <sup>bcd</sup>	93.97 ± 0.73 <sup>bc</sup>
US - 20min		1.52 ± 0.06 <sup>abc</sup>	11.13 ± 1.91 <sup>de</sup>	92.36 ± 0.31 <sup>abc</sup>	94.51 ± 0.37 <sup>bc</sup>
US - 30min		1.48 ± 0.02 <sup>abc</sup>	14.15 ± 2.76 <sup>cde</sup>	92.92 ± 0.74 <sup>abc</sup>	95.21 ± 0.59 <sup>abc</sup>
US - 40min		1.38 ± 0.09 <sup>c</sup>	28.02 ± 9.58 <sup>a</sup>	93.72 ± 1.23 <sup>ab</sup>	95.22 ± 0.57 <sup>abc</sup>
Control	45 °C	1.51 ± 0.04 <sup>ab</sup>	16.25 ± 2.39 <sup>bcd</sup>	89.72 ± 0.85 <sup>cd</sup>	93.78 ± 0.37 <sup>bc</sup>
US - 10min		1.49 ± 0.02 <sup>abc</sup>	23.29 ± 2.53 <sup>abc</sup>	91.38 ± 1.65 <sup>abcd</sup>	94.61 ± 0.86 <sup>abc</sup>
US - 20min		1.42 ± 0.01 <sup>bc</sup>	30.97 ± 1.37 <sup>a</sup>	93.20 ± 0.61 <sup>abc</sup>	96.00 ± 0.35 <sup>ab</sup>
US - 30min		1.40 ± 0.02 <sup>bc</sup>	28.15 ± 1.05 <sup>a</sup>	93.85 ± 0.41 <sup>a</sup>	97.13 ± 2.28 <sup>a</sup>
US - 40min		1.38 ± 0.01 <sup>c</sup>	25.44 ± 1.13 <sup>ab</sup>	94.51 ± 1.26 <sup>a</sup>	95.98 ± 0.40 <sup>ab</sup>

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:  $T_{\text{hydration}}: 25^{\circ}\text{C}$ ; D:  $T_{\text{hydration}}: 45^{\circ}\text{C}$ ) 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).

### **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 °C (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 <sup>a</sup>	22.8 ± 0.2 <sup>f</sup>	35.9 ± 0.1 <sup>g</sup>	33.7 ± 0.3 <sup>a</sup>	3.65 ± 0.08 <sup>a</sup>
Control		3.90 ± 0.07 <sup>b</sup>	25.3 ± 0.4 <sup>e</sup>	36.9 ± 0.6 <sup>fg</sup>	30.2 ± 1.0 <sup>b</sup>	3.64 ± 0.09 <sup>a</sup>
US - 10min	25 °C	3.90 ± 0.04 <sup>ab</sup>	26.2 ± 0.4 <sup>d</sup>	37.5 ± 0.6 <sup>def</sup>	28.7 ± 0.7 <sup>cd</sup>	3.65 ± 0.08 <sup>a</sup>
US - 20min		3.87 ± 0.02 <sup>b</sup>	26.5 ± 0.3 <sup>d</sup>	38.2 ± 0.8 <sup>cde</sup>	27.8 ± 0.8 <sup>de</sup>	3.63 ± 0.06 <sup>a</sup>
US - 30min		3.93 ± 0.05 <sup>ab</sup>	27.1 ± 0.3 <sup>bc</sup>	38.9 ± 0.6 <sup>abc</sup>	26.5 ± 0.5 <sup>fg</sup>	3.63 ± 0.08 <sup>a</sup>
US - 40min		3.91 ± 0.07 <sup>ab</sup>	27.6 ± 0.3 <sup>b</sup>	39.1 ± 0.4 <sup>abc</sup>	25.7 ± 0.6 <sup>g</sup>	3.63 ± 0.06 <sup>a</sup>
Control		3.88 ± 0.01 <sup>b</sup>	25.5 ± 0.2 <sup>e</sup>	37.1 ± 0.6 <sup>ef</sup>	29.9 ± 0.4 <sup>bc</sup>	3.65 ± 0.09 <sup>a</sup>
US - 10min	45 °C	3.90 ± 0.05 <sup>b</sup>	26.2 ± 0.2 <sup>d</sup>	37.8 ± 0.5 <sup>def</sup>	28.5 ± 0.6 <sup>de</sup>	3.63 ± 0.09 <sup>a</sup>
US - 20min		3.83 ± 0.08 <sup>b</sup>	26.8 ± 0.2 <sup>cd</sup>	38.4 ± 0.6 <sup>bcd</sup>	27.4 ± 0.5 <sup>ef</sup>	3.61 ± 0.07 <sup>a</sup>
US - 30min		3.84 ± 0.02 <sup>b</sup>	27.3 ± 0.3 <sup>bc</sup>	39.3 ± 0.6 <sup>ab</sup>	25.9 ± 0.6 <sup>g</sup>	3.64 ± 0.08 <sup>a</sup>
US - 40min		3.88 ± 0.08 <sup>b</sup>	28.2 ± 0.2 <sup>a</sup>	40.0 ± 0.7 <sup>a</sup>	24.3 ± 0.7 <sup>h</sup>	3.63 ± 0.08 <sup>a</sup>

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

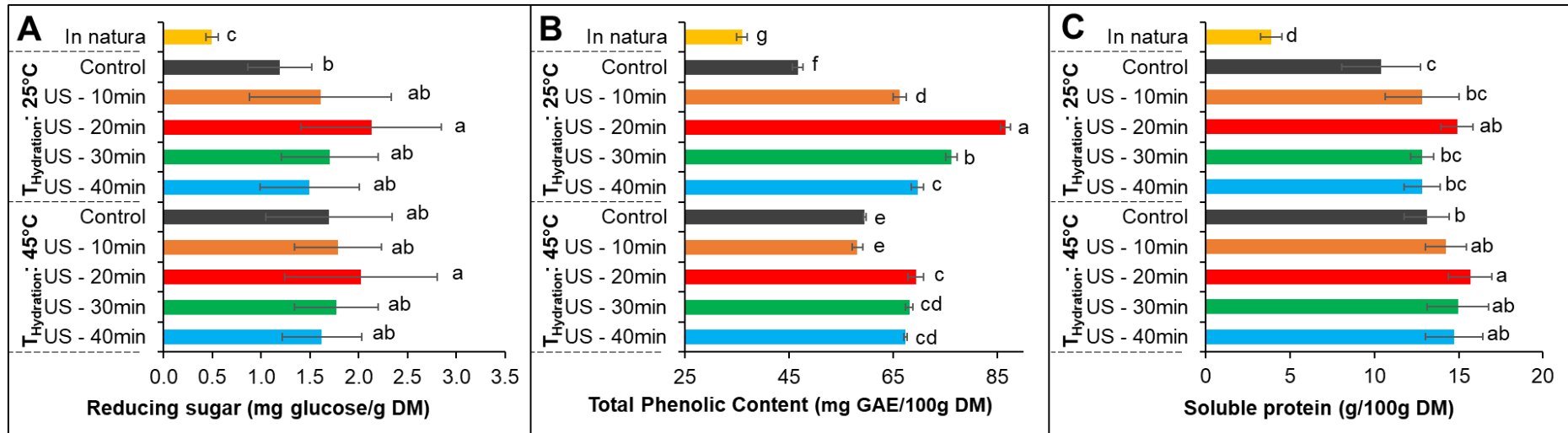
Fig. 5 presents the results regarding the impact of ultrasound-assisted hydration at 25 °C and 45 °C on the concentration of reducing sugars (A), total phenolic content (B), and soluble protein content (C) in pumpkin seed flour germinated at 25 °C for 72 h.

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  $15.7 \pm 1.3$  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 sprouting 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).

## **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 germinated 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.

**Author Contributions** Flaviana Coelho Pacheco: Conceptualization, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing. Jeferson Silva Cunha: Formal analysis, Investigation. Irene Andressa: Formal analysis, Investigation. Fábio Ribeiro dos Santos: Formal analysis, Investigation. Ana Flávia Coelho Pacheco: Conceptualization, Formal analysis, Investigation, Writing - Original Draft. Gabriela Aparecida Nalon: Formal analysis, Investigation. Paulo Henrique Costa Paiva: Conceptualization, Investigation, Writing - Original Draft. Alline Artigiani Lima Tribst: Conceptualization, Methodology, Visualization, Writing - Original Draft, Writing - Review & Editing. Pedro Esteves Duarte Augusto: Visualization, Writing - Original Draft, Writing - Review & Editing. Bruno Ricardo de Castro Leite Júnior: Conceptualization, Methodology, Formal analysis, Resources, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

**Funding** The authors are grateful to the Coordination of Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001 and the Minas Gerais Research Foundation (FAPEMIG, Brazil) for funding the project APQ-00388-21/APQ-00785-23. Communauté Urbaine du Grand Reims, Département de la Marne, Région Grand Est and European Union (FEDER Champagne-Ardenne 2014-2020, FEDER Grand Est 2021-2027) are acknowledged for their financial support to the Chair of Biotechnology of CentraleSupélec and the Centre Européen de Biotechnologie et de Bioéconomie (CEBB).

**Data Availability** No datasets were generated or analysed during the current study.

## **Declarations**

**Competing Interests** The authors declare no competing interests.

## References

- Aires, M. R., Balbinoti, T. C. V., Maciel, G. M., Fernandes, I. D. A. A., Jorge, R. M. M., & Haminiuk, C. W. I. (2023). Enhancing the parboiling of pantanal rice with ultrasound-assisted hydration: Mass transfer kinetics and bioactive properties. *Journal of Cereal Science*, *114*, 103762. <https://doi.org/10.1016/j.jcs.2023.103762>.
- Ampofo, J. O., & Ngadi, M. (2020). Ultrasonic assisted phenolic elicitation and antioxidant potential of common bean (*Phaseolus vulgaris*) sprouts. *Ultrasonics Sonochemistry*, *64*, 104974. <https://doi.org/10.1016/j.ultsonch.2020.104974>.
- Andressa, I., Nascimento, G.K.S., Santos, T.M., Oliveira, J. R., Benassi, V.M., & Schmiele, M. (2023). Alterações físico-químicas induzidas por processo germinativo de sementes de milho de pericarpo roxo. *Brazilian Journal of Development*, *9*(2), 8051–8070. <https://doi.org/10.34117/BJDV9N2-125>
- Atudorei, D., Stroe, S. G., & Codină, G. G. (2021). Impact of germination on the microstructural and physicochemical properties of different legume types. *Plants*, *10*(3), 592. <https://doi.org/10.3390/plants10030592>.
- AOAC, 2005. Official Methods of Analysis of the Association of Official Analytical Chemists International, 18th ed. AOAC, Washington, DC.
- Balbinoti, T. C. V., de Matos Jorge, L. M., Haminiuk, C. W. I., & Jorge, R. M. M. (2022). Multiphysics simulation and characterisation of parboiling of long grain rice during hydration. *Journal of Cereal Science*, *103*, 103391. <https://doi.org/10.1016/j.jcs.2021.103391>.
- Bewley, J. D., Bradford, K., & Hilhorst, H. (2012). Seeds: physiology of development, germination and dormancy. *Springer Science & Business Media*.
- Bryce, J. H., Goodfellow, V., Agu, R. C., Brosnan, J. M., Bringhurst, T. A., & Jack, F. R. (2010). Effect of different steeping conditions on endosperm modification and quality of distilling malt.



*Journal of the Institute of Brewing*, 116(2), 125-133. <https://doi.org/10.1002/j.2050-0416.2010.tb00408.x>.

Carvalho, G. R., Rojas, M. L., de Oliveira Gomes, B., & Augusto, P. E. D. (2024). Emerging approaches to improve barley malt processing and quality: ultrasound-assisted hydration and ethanol pre-treatment to drying. *Journal of Food Engineering*, 112098. <https://doi.org/10.1016/j.jfoodeng.2024.112098>.

Chiu, K. Y., & Sung, J. M. (2014). Use of ultrasonication to enhance pea seed germination and microbial quality of pea sprouts. *International Journal of Food Science & Technology*, 49(7), 1699-1706. <https://doi.org/10.1111/ijfs.12476>.

Chiu, K. Y. (2021). Changes in microstructure, germination, sprout growth, phytochemical and microbial quality of ultrasonication treated adzuki bean seeds. *Agronomy*, 11(6), 1093. <https://doi.org/10.3390/agronomy11061093>.

Corzo, O., Bracho, N., & Alvarez, C. (2010). Weibull model for thin-layer drying of mango slices at different maturity stages. *Journal of Food Processing and Preservation*, 34(6), 993-1008. <https://doi.org/10.1111/j.1745-4549.2009.00433.x>.

Cosson, A., Correia, L. O., Descamps, N., Saint-Eve, A., & Souchon, I. (2022). Identification and characterization of the main peptides in pea protein isolates using ultra high-performance liquid chromatography coupled with mass spectrometry and bioinformatics tools. *Food Chemistry*, 367, 130747. <https://doi.org/10.1016/j.foodchem.2021.130747>

Ding, J., Johnson, J., Chu, Y. F., & Feng, H. (2019). Enhancement of  $\gamma$ -aminobutyric acid, avenanthramides, and other health-promoting metabolites in germinating oats (*Avena sativa* L.) treated with and without power ultrasound. *Food Chemistry*, 283, 239-247. <https://doi.org/10.1016/j.foodchem.2018.12.136>.

- do Nascimento, L. Á., Abhilasha, A., Singh, J., Elias, M. C., & Colussi, R. (2022). Rice germination and its impact on technological and nutritional properties: A review. *Rice Science*, 29(3), 201-215. <https://doi.org/10.1016/j.rsci.2022.01.009>
- Ebert, A. W. (2022). Sprouts and microgreens—novel food sources for healthy diets. *Plants*, 11(4), 571. <https://doi.org/10.3390/plants11040571>
- Farzaneh, V., Ghodsvali, A., Bakhshabadi, H., Zare, Z., & Carvalho, I. S. (2017). The impact of germination time on the some selected parameters through malting process. *International Journal of Biological Macromolecules*, 94, 663-668. <https://doi.org/10.1016/j.ijbiomac.2016.10.052>.
- Finnie, S., Brovelli, V., & Nelson, D. (2019). *Sprouted Grains as a Food Ingredient Sprouted Grains*. Elsevier: Amsterdam, The Netherlands, pp. 113–142.
- Ghaderi, F. A., Soltani, A., & Sadeghipour, H. R. (2008). Cardinal temperatures of germination in medicinal pumpkin (*Cucurbita pepo convar. pepo var. styriaca*), borago (*Borago officinalis* L.) and black cumin (*Nigella sativa* L.). *Asian Journal of Plant Sciences*, 7, 574-578. <https://doi.org/10.3923/ajps.2008.574.578>
- Guimarães, B., Polachini, T. C., Augusto, P. E., & Telis-Romero, J. (2020). Ultrasound-assisted hydration of wheat grains at different temperatures and power applied: Effect on acoustic field, water absorption and germination. *Chemical Engineering and Processing-Process Intensification*, 155, 108045. <https://doi.org/10.1016/j.cep.2020.108045>.
- Huang, S., Ashraf, U., Duan, M., Ren, Y., Xing, P., Yan, Z., & Tang, X. (2024). Ultrasonic seed treatment improved seed germination, growth, and yield of rice by modulating associated physio-biochemical mechanisms. *Ultrasonics Sonochemistry*, 104, 106821. <https://doi.org/10.1016/j.ultsonch.2024.106821>.
- Jideani, V. A., & Mpotokwana, S. M. (2009). Modeling of water absorption of Botswana bambara varieties using Peleg's equation. *Journal of Food Engineering*, 92(2), 182-188. <https://doi.org/10.1016/j.jfoodeng.2008.10.040>.

- Kalita, D., Jain, S., Srivastava, B., & Goud, V. V. (2021). Sono-hydro priming process (ultrasound modulated hydration): Modelling hydration kinetic during paddy germination. *Ultrasonics Sonochemistry*, *70*, 105321. <https://doi.org/10.1016/j.ultsonch.2020.105321>
- Kumar, G., Le, D. T., Durco, J., Cianciosi, S., Devkota, L., & Dhital, S. (2023). Innovations in legume processing: Ultrasound-based strategies for enhanced legume hydration and processing. *Trends in Food Science & Technology*, *139*, 104122. <https://doi.org/10.1016/j.tifs.2023.104122>
- Lemmens, E., Moroni, A. V., Pagand, J., Heirbaut, P., Ritala, A., Karlen, Y., ... & Delcour, J. A. (2019). Impact of cereal seed sprouting on its nutritional and technological properties: A critical review. *Comprehensive Reviews in Food Science and Food Safety*, *18*(1), 305-328. <https://doi.org/10.1111/1541-4337.12414>.
- Li, S., Luo, Z., Guan, X., Huang, K., Li, Q., Zhu, F., & Liu, J. (2019). Effect of ultrasonic treatment on the hydration and physicochemical properties of brewing rice. *Journal of Cereal Science*, *87*, 78-84. <https://doi.org/10.1016/j.jcs.2019.03.002>.
- Liu, H., Li, Z., Zhang, X., Liu, Y., Hu, J., Yang, C., & Zhao, X. (2021). The effects of ultrasound on the growth, nutritional quality and microbiological quality of sprouts. *Trends in Food Science & Technology*, *111*, 292-300. <https://doi.org/10.1016/j.tifs.2021.02.065>.
- Liu, M., Childs, M., Loos, M., Taylor, A., Smart, L. B., & Abbaspourrad, A. (2023). The effects of germination on the composition and functional properties of hemp seed protein isolate. *Food Hydrocolloids*, *134*, 108085. <https://doi.org/10.1016/j.foodhyd.2022.108085>.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*(1), 265-275.
- Louf, J. F., Zheng, Y., Kumar, A., Bohr, T., Gundlach, C., Harholt, J., ... & Jensen, K. H. (2018). Imbibition in plant seeds. *Physical Review E*, *98*(4), 042403. <https://doi.org/10.1103/PhysRevE.98.042403>.

- Miano, A. C., Pereira, J. D. C., Castanha, N., Júnior, M. D. D. M., & Augusto, P. E. D. (2016). Enhancing mung bean hydration using the ultrasound technology: description of mechanisms and impact on its germination and main components. *Scientific Reports*, 6(1), 38996. <https://doi.org/10.1038/srep38996>.
- Miano, A. C., Ibarz, A., & Augusto, P. E. D. (2017). Ultrasound technology enhances the hydration of corn kernels without affecting their starch properties. *Journal of Food Engineering*, 197, 34-43. <https://doi.org/10.1016/j.jfoodeng.2016.10.024>.
- Miano, A. C., & Augusto, P. E. D. (2018). The hydration of grains: A critical review from description of phenomena to process improvements. *Comprehensive Reviews in Food Science and Food Safety*, 17(2), 352-370. <https://doi.org/10.1111/1541-4337.12328>.
- Miano, A. C., Saldaña, E., Campestrini, L. H., Chiorato, A. F., & Augusto, P. E. D. (2018). Correlating the properties of different carioca bean cultivars (*Phaseolus vulgaris*) with their hydration kinetics. *Food Research International*, 107, 182-194. <https://doi.org/10.1016/j.foodres.2018.02.030>
- Miano, A. C., Rojas, M. L., & Augusto, P. E. (2019). Using ultrasound for improving hydration and debittering of Andean lupin grains. *Journal of Food Process Engineering*, 42(6), e13170. <https://doi.org/10.1111/jfpe.13170>.
- Naumenko, N., Potoroko, I., & Kalinina, I. (2022). Stimulation of antioxidant activity and  $\gamma$ -aminobutyric acid synthesis in germinated wheat grain *Triticum aestivum* L. by ultrasound: Increasing the nutritional value of the product. *Ultrasonics Sonochemistry*, 86, 106000. <https://doi.org/10.1016/j.ultsonch.2022.106000>.
- Ninčević Grassino, A., Rimac Brnčić, S., Badanjak Sabolović, M., Šic Žlabur, J., Marović, R., & Brnčić, M. (2023). Carotenoid content and profiles of pumpkin products and by-products. *Molecules*, 28(2), 858. <https://doi.org/10.3390/molecules28020858>.

- Noh, N. A. N. M., Karim, L., & Omar, S. R. (2022). Value-added products from pumpkin wastes: a review. *Malaysian Journal of Science Health & Technology*, 8(1), 77-84. <https://doi.org/10.33102/mjosht.v8i1.231>.
- Patero, T., & Augusto, P. E. (2015). Ultrasound (US) enhances the hydration of sorghum (*Sorghum bicolor*) grains. *Ultrasonics Sonochemistry*, 23, 11-15. <https://doi.org/10.1016/j.ultsonch.2014.10.021>
- Pacheco, A. F. C., Pacheco, F. C., Cunha, J. S., dos Santos, F. R., Pacheco, J. C. C., de Paula Correa, K., ... & Leite Junior, B. R. C. (2024). Bibliometric analysis of pumpkin seed proteins: A review of the multifunctional properties of their hydrolysates and future perspectives. *Food Bioscience*, 59, 104269. <https://doi.org/10.1016/j.fbio.2024.104269>
- Pesti-Asbóth, G., Molnár-Bíró, P., Forgács, I., Remenyik, J., & Dobránszki, J. (2022). Ultrasonication affects the melatonin and auxin levels and the antioxidant system in potato in vitro. *Frontiers in Plant Science*, 13, 979141. <https://doi.org/10.3389/fpls.2022.979141>.
- Pilco-Quesada, S., Tian, Y., Yang, B., Repo-Carrasco-Valencia, R., & Suomela, J. P. (2020). Effects of germination and kilning on the phenolic compounds and nutritional properties of quinoa (*Chenopodium quinoa*) and kiwicha (*Amaranthus caudatus*). *Journal of Cereal Science*, 94, 102996. <https://doi.org/10.1016/j.jcs.2020.102996>.
- Polachini, T. C., Norwood, E. A., Le-Bail, P., Le-Bail, A., & Cárcel, J. A. (2023). Pulsed electric field (PEF) application on wheat malting process: Effect on hydration kinetics, germination and amylase expression. *Innovative Food Science & Emerging Technologies*, 86, 103375. <https://doi.org/10.1016/j.ifset.2023.103375>.
- Raso, J., Manas, P., Pagan, R., & Sala, F. J. (1999). Influence of different factors on the output power transferred into medium by ultrasound. *Ultrasonics Sonochemistry*, 5(4), 157-162. [https://doi.org/10.1016/S1350-4177\(98\)00042-X](https://doi.org/10.1016/S1350-4177(98)00042-X).

- Saavedra, M. J., Aires, A., Dias, C., Almeida, J. A., De Vasconcelos, M. C. B. M., Santos, P., & Rosa, E. A. (2015). Evaluation of the potential of squash pumpkin by-products (seeds and shell) as sources of antioxidant and bioactive compounds. *Journal of Food Science and Technology*, 52, 1008-1015. <https://doi.org/10.1007/s13197-013-1089-5>.
- Saqib, A. A. N., & Whitney, P. J. (2011). Differential behaviour of the dinitrosalicylic acid (DNS) reagent towards mono-and di-saccharide sugars. *Biomass and Bioenergy*, 35(11), 4748-4750. <https://doi.org/10.1016/j.biombioe.2011.09.013>.
- Sharma, S., Saxena, D. C., & Riar, C. S. (2017). Using combined optimization, GC–MS and analytical technique to analyze the germination effect on phenolics, dietary fibers, minerals and GABA contents of Kodo millet (*Paspalum scrobiculatum*). *Food Chemistry*, 233, 20-28. <https://doi.org/10.1016/j.foodchem.2017.04.099>
- Shen, P. (2024). Plant-Based Protein Flavor Maskers and Enhancers. *Flavor-Associated Applications in Health and Wellness Food Products*, 321-344. [https://doi.org/10.1007/978-3-031-51808-9\\_13](https://doi.org/10.1007/978-3-031-51808-9_13)
- Singh, A. K., Rehal, J., Kaur, A., & Jyot, G. (2015). Enhancement of attributes of cereals by germination and fermentation: A review. *Critical Reviews in Food Science and Nutrition*, 55(11), 1575-1589. <https://doi.org/10.1080/10408398.2012.706661>
- Singh, A., & Kumar, V. (2023). Pumpkin seeds as nutraceutical and functional food ingredient for future: A review. *Grain & Oil Science and Technology*, 7, 12-29. <https://doi.org/10.1016/j.gaost.2023.12.002>
- Silva, J. A. T., & Dobránszki, J. (2014). Sonication and ultrasound: impact on plant growth and development. *Plant Cell, Tissue and Organ Culture*, 117, 131-143.
- Silva, R.F., Pogačnik, L. (2020). Polyphenols from food and natural products: Neuroprotection and safety. *Antioxidants*, 9(1), 61.
- Sun, X., Chen, H., Wang, P., Chen, F., Yuan, L., & Mi, G. (2020). Low nitrogen induces root elongation via auxin-induced acid growth and auxin-regulated target of rapamycin (TOR)

- pathway in maize. *Journal of Plant Physiology*, 254, 153281. <https://doi.org/10.1016/j.jplph.2020.153281>.
- Tan, M., Nawaz, M. A., & Buckow, R. (2023). Functional and food application of plant proteins—a review. *Food Reviews International*, 39(5), 2428-2456. <https://doi.org/10.1080/87559129.2021.1955918>
- Xiang, J., Yuan, Y., Du, L., Zhang, Y., Li, C., & Beta, T. (2023). Modification on phenolic profiles and enhancement of antioxidant activity of proso millets during germination. *Food Chemistry: X*, 18, 100628. <https://doi.org/10.1016/j.fochx.2023.100628>.
- Yaldagard, M., Mortazavi, S. A., & Tabatabaie, F. (2008). Influence of ultrasonic stimulation on the germination of barley seed and its alpha-amylase activity. *African Journal of Biotechnology*, 7(14).
- Yang, H., Gao, J., Yang, A., & Chen, H. (2015). The ultrasound-treated soybean seeds improve edibility and nutritional quality of soybean sprouts. *Food research international*, 77, 704-710. <https://doi.org/10.1016/j.foodres.2015.01.011>
- Yıldırım, A. (2022). Influence of temperature, ultrasound, and variety on moisture diffusivity and thermodynamic properties of some durum wheat varieties during hydration. *Journal of Food Processing and Preservation*, 46(4), e16463. <https://doi.org/10.1111/jfpp.16463>
- Zhang, Q., Liu, Y., Yu, Q., Ma, Y., Gu, W., & Yang, D. (2020). Physiological changes associated with enhanced cold resistance during maize (*Zea mays*) germination and seedling growth in response to exogenous calcium. *Crop and Pasture Science*, 71(6), 529-538. <https://doi.org/10.1071/CP19510>
- Zhang, H., Mo, W., Liao, S., Jia, Z., Zhang, W., Zhang, S., & Liu, Z. (2023). Ultrasound promotes germination of aging *Pinus tabuliformis* seeds is associated with altered lipid metabolism. *Ultrasonics Sonochemistry*, 93, 106310. <https://doi.org/10.1016/j.ultsonch.2023.106310>
- Zehtab-Salmasi, S. (2006). Study of cardinal temperatures for pumpkin (*Cucurbita pepo*) seed germination. *Journal of Agronomy*, 5, 95-97. <https://doi.org/10.3923/ja.2006.95.97>.

Zwietering, M. H., Jongenburger, I., Rombouts, F. M., & Van't Riet, K. J. A. E. M. (1990). Modeling of the bacterial growth curve. *Applied and Environmental Microbiology*, 56(6), 1875-1881. <https://doi.org/10.1128/aem.56.6.1875-1881.1990>.