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Optimization of ultrasound-assisted hydration of oat seeds: Effects of amplitude and exposure time on water absorption, germination, and antioxidant capacity

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ORIGINAL ARTICLE

Abstract

Ultrasound (US) technology is a nonthermal technique for enhancing grain hydration properties. In this study, oat seeds were treated with US at different amplitudes (20, 30, and 40%) and exposure times (20, 30, and 40 min). The effects of germination time (30, 36, 42, and 48 h) were evaluated in both US-treated and untreated oat seeds by measuring the water absorption kinetics, germination rate, root length, imbibition weight, total phenolic content (TPC), antioxidant activity, and beta-glucan content. The Weibull model best described the soaking kinetics of oat seeds across the investigated amplitudes and ultrasonic durations owing to the highest R^2 (0.98–0.99) and the lowest standard errors (0.013–0.028). The results demonstrated that US treatment enhanced both hydration and bioactive component levels. Compared to the control, the optimized oats germinated through US treatment had approximately 220% and 64% increase in TPC and DPPH scavenging activity, respectively. Germination times of 42–48 h favor germination rate and antioxidant properties. The recommended conditions for producing germinated oats with optimal properties and high TPC, DPPH, and β-glucan are 30% US amplitude, 40 min of sonication, and 42 h of germination. This technique enhanced the nutritional and functional properties of seeds germinated through US, supporting their potential use in functional foods, nutraceuticals, and fortified grain products.

Keywords: Ultrasound technology; Germination; Oat; Antioxidant; Hydration; β-glucan

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Introduction

Oats (*Avena sativa* L.) are a nutrient-rich cereal crop valued for their high β-glucan content, essential amino acids, unsaturated fatty acids, and unique polyphenols such as avenanthramides, which exhibit antioxidant and anti-inflammatory properties (Paudel *et al.*, 2021). Despite these benefits, conventional germination methods often result in inconsistent bioactive compound profiles, prolonged soaking durations, and limited functional enhancement, thereby constraining their industrial and nutritional application.

Recent advancements in nonthermal technologies have introduced ultrasound (US) treatment as a promising alternative for improving grain hydration and germination. US promotes rapid water absorption, disrupts seed coat barriers, and stimulates metabolic activity via cavitation-induced mechanical effects, resulting in enhanced seed viability and bioactive compound synthesis (Estivi *et al.*, 2022; Gong *et al.*, 2024). Compared to traditional soaking, US-assisted germination can reduce processing time and increase the yield of health-promoting metabolites.

However, the response of oat seeds—especially locally grown cultivars—to varied US parameters such as amplitude and exposure time remains underexplored. There is limited empirical data on how US affects hydration kinetics, germination efficiency, and antioxidant profiles in oats. Thus, a systematic investigation is necessary to determine optimal US conditions for maximizing nutritional quality and functional value.

Seed germination is a critical stage in plant development and a key determinant of overall plant productivity. It involves water absorption, food reserve mobilization, protein synthesis, and radicle emergence, triggering structural changes and the production of bioactive compounds that enhance the nutritional value and stability of grains (Ali and Elozeiri, 2017). During this process, structural modification and the development of new compounds, many of which have high bioactivity, can increase the nutritional value and stability of grains (Liu *et al.*, 2022). Statistical modeling supports the prediction and optimization of seed hydration behavior, helping to select appropriate soaking parameters for improved germination efficiency (Ansari *et al.*, 2015). According to Resende and Corrêa (2007), these tools are essential for simulating the behavior of materials subjected to the hydration process and can use theoretical and empirical models. Currently, various technologies are available to enhance seed germination, such as US, ultraviolet (UV) light, and magnetic fields. Different techniques can induce various changes in seed germination. Some techniques lead to physiological changes, hormonal changes, and the destruction of plant structures because of various factors (Rifna *et al.*, 2019).

US is a mechanical wave that requires an elastic medium for its propagation (Lempriere, 2003; Mason and Peters, 2002). Energy transfer occurs through longitudinal and transverse waves, each propagating differently, depending on the medium. Longitudinal waves can travel through any medium, whereas transverse waves are limited to solids (Rose, 2000; Zhou *et al.*, 2021). US, which utilizes sound waves in the US frequency range (typically between 20 kHz and 100 kHz), has been shown to enhance absorption and improve yield in various food-processing applications while reducing energy consumption. More recently, US treatment has gained attention as a promising, eco-friendly technique to enhance seed germination and early seedling development. By promoting water absorption, disrupting seed coat barriers, and modifying biochemical components such as starch and proteins, ultrasonication can significantly improve germination rates, seed vigor, and plant growth. These benefits make US a valuable tool in sustainable agriculture and seed technology (Gong *et al.*, 2024). This technique affects cereal seed germination via biological, chemical, and physical methods (Yaldagard *et al.*, 2008). The mechanisms involve transferring energy mechanically, increasing the surface pores, and cracking to promote hydration in seeds. However, owing to the sparse and dense nature of US applications, it can destroy cells, making way for water molecules (cavitation effect). It has been reported that ultrasonication can enhance the stimulation and germination of cereal seeds by changing the starch content to a higher amylose content, water solubility, swelling power, and viscosity (Bai *et al.*, 2023). In addition, Estivi *et al.* (2022) noted that US treatment can modify the structure of macromolecules in cereals and pseudocereals, such as starch and proteins, often improving their technological, functional, and bioactive properties. The enhancement of γ-aminobutyric acid, avenanthramides, and other health-promoting metabolites in germinating oats *(Avena sativa L.)* treated with and without power US was also studied by Ding *et al.* (2019). US-assisted germination has gained interest in the food industry because of its potential to enhance the nutritional, functional, and processing properties of seeds, making them more suitable for applications in health-oriented food products, functional flours, and plant-based formulations.

Conventional germination methods of oats often require prolonged soaking and produce inconsistent bioactive compound profiles. These limitations hinder processing efficiency and functional food development. US-assisted germination overcomes these drawbacks by accelerating hydration, improving enzymatic activity, and enhancing the release of bioactive compounds. This positions US as a promising, nonthermal approach to optimize oat germination. In addition, the effects of US on oat seeds grown in Thailand may be similar to, or different from, those on other cereals. Therefore, in order to promote the utilization and increase values of home grown oat using US pre-tretment to produce value added-germinated oat, it is necessary to study the germination of oats of varying amplitudes and durations. Hydration kinetics were investigated to determine the appropriate model associated with the soaking step. This study also explored the effect of US treatment on the germination and antioxidant properties of germinated oats.

Material and Methods

Chemicals

All reagents used in this study were of analytical grade. Methanol (≥99.8%, analytical grade), sodium carbonate (Na_2CO_3, ≥99.5%), and Folin–Ciocalteu reagent were purchased from Merck KGaA (Darmstadt, Germany). DPPH (2,2-diphenyl-1-picrylhydrazyl, ≥95%) and Trolox standard (≥97%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The β-glucan measurement kit (K-BGLU) was supplied by Megazyme International Ireland Ltd. (Bray, Ireland). All solutions were prepared using deionized water (resistivity ≥18.2 MΩ·cm) from a Milli-Q system (Millipore, USA).

Samples collection and treatment procedures

Oat grains (*Avena sativa L., 1753*) grown at the Samoeng Rice Center, Chiang Mai, Thailand, are members of the genus *Avena* within the grass family (Poaceae), with 6.01% moisture content. The moisture content was measured by the authors using the oven-drying method (AOAC 925.10), where oat grains were dried at 105°C for 24 h until a constant weight was achieved. Dried samples were collected in zipped bags and stored at 25°C.

For ultrasonic experiment, 80 g of oats was mixed with 300 mL of distilled water (1:4 w/v) and divided into two groups: one soaked without US and the other treated ultrasonically (500 W, 30°C) for 20, 30, or 40 min at amplitudes of 20%, 30%, and 40%, respectively. The control samples were ultrasonically left untreated. The ultrasonic sonicator (VCX 134ATA, Sonics & Materials, Inc. Connecticut, USA) was conducted in a temperature-controlled water bath maintained at 30°C.

The sonication temperature was maintained at 30°C to balance cavitation efficiency with enzyme preservation. At this moderate temperature, cavitation effects remain strong enough to enhance hydration and disrupt seed structures, while minimizing thermal degradation or inactivation of germination-related enzymes such as amylases and proteases. Temperatures above 35°C could accelerate enzyme denaturation or initiate premature sprouting, whereas lower temperatures could reduce cavitation intensity and hydration kinetics (Estivi *et al.*, 2022; Gong *et al.*, 2024). Thus, 30°C was selected as an optimal compromise for effective US-assisted germination. This helped prevent overheating, ensuring consistent cavitation effects while preserving enzymatic activity. Minor fluctuations (<2°C) were monitored but did not affect the outcome.

The amplitudes of 20%, 30%, and 40% were selected based on previous studies showing their efficacy in enhancing hydration and biochemical modifications in grains (Ding *et al.*, 2019; Estivi *et al.*, 2022). These levels represent low to moderate ultrasonic intensities where cavitation effects can improve permeability without inducing cell damage. Our findings show that 30% amplitude provides an optimal balance, significantly enhancing germination and antioxidant properties without structural degradation.

To perform the hydration kinetic study, after US treatment, the oats were soaked in water for 10 h, and the details are explained below.

After soaking, both groups were germinated for 30, 36, 42, or 48 h, and then subjected to drying. The drying time at 52°C was approximately 16 h, until the grain moisture content fell below 14%. The dried samples were then ground and passed through a 60-mesh sieve, yielding powder with a particle size range of approximately 250–300 μm. Analyses were conducted to evaluate the root length and germination rate. The antioxidant capacity of samples from both groups of germinated oats (US-treated and untreated), which were dried after germination times of 30, 36, 42, and 48 h, was assessed. The dry samples were ground into powder for the analysis of phenolic content, DPPH radical scavenging activity, and β-glucan content.

Hydration procedure

Following ultrasonic treatment, the oats were drained to remove excess water and subjected to a water-absorption test. Each sample was weighed and placed in a water box for absorption measurements. The increasing weight of the oats was recorded at specific intervals (hourly) until saturation, as indicated by a constant weight. Thereafter, the hydration kinetics of oat seeds under different US conditions were evaluated to obtain the primary models using Peleg, Weibull, first-order, and exponential models. Consequently, the selected models were assessed using empirical mathematical relationships derived as functions of process parameters.

Germination rate

The germination experiment was conducted at 27 ± 1°C in a plastic box with a moistened paper towel layer as the substrate. After treatment with or without US and soaking at room temperature, samples were placed in a closed cabinet for 30, 36, 42, and 48 h. Seeds were considered germinated when rootlets appeared at the edge of the seed. Water was added as required to maintain moisture. The germination rate was calculated using the following formula:

$$\text{Germination rate (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100 \tag{1}$$

Root length

After germination for 30, 36, 42, or 48 h, the root length of the oat seeds was measured using a Vernier caliper (Winton, Gammaco Thailand Co., Ltd.), and the results were recorded in millimeters (mm).

Extraction and determination of total phenolic compounds

Samples (0.5 g of powder) were extracted using 40 mL of methanol/water (50% v/v). The mixture was shaken for 30 min at 27 ± 1°C and filtered through a filter paper. The total phenolic content (TPC) of the extracts was determined using the Folin–Ciocalteu spectrophotometric method (Singleton *et al.*, 1999). To 0.5 mL of extract, 2.5 mL of 10% Folin–Ciocalteu reagent was added and incubated for 5 min in the dark, followed by 2 mL of 7.5% Na_2CO_3 solution. The mixture was then incubated for 30 min in the dark at room temperature. Absorbance was measured at 760 nm (Genesys 10S UV-VIS, Thermo Scientific, USA). TPC was expressed as gallic acid equivalent (GAE) per 100 g dry weight (DW) of the grain material.

Determination of antioxidant activity

The antioxidant activity of the oat seed extracts was evaluated using the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, following the method described by Yu *et al.* (2003). To 1.5 mL of extract, 1.5 mL of 0.1 mM DPPH solution was added, and the mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm. The results are expressed as Trolox equivalents (TE) per 100 g DW of the grain material.

Measurement of β-glucan content

The germinated oat grains were then ground. The β-glucan content was measured using an enzyme kit (Megazyme, Bray Town, Ireland). A 0.1 g powder sample was mixed with 0.2 mL of 50% ethanol. Sodium Phosphate buffer (pH 6.5; 4 mL of 20 mM) was added to maintain a stable pH for enzymatic reactions. The mixture was heated to 100°C for 1 min and vortexed for 1 min to ensure uniform mixing. For enzymatic digestion, the sample was incubated at 50°C for 1 h, after 0.2 mL of B1 (lichenase in PP buffer) was added, and the mixture was incubated in the dark at 50°C for 5 min. The mixture was then heated at 100°C for 2 min to inactivate lichenase, followed by 2 min of vortexing to ensure complete inactivation. After adding 5 mL of 200 mM Sodium Acetate buffer (pH 4.0), a magnetic stirrer bar was added, and the mixture was centrifuged (Z206A, Hermle, Germany) at 5000 rpm for 10 min. A 0.1 mL aliquot of the supernatant was collected, mixed with 0.1 mL of B2 (β-Glucosidase), and incubated at 50°C for 10 min. The GOPOD reagent (3 mL) was added and incubated at 50°C for 20 min to quantify glucose via the GOPOD reaction. For controls or standards, 0.1 mL of 50 mM acetate buffer (pH 4.0) and 0.1 mL of B5 (D-glucose) were added. Finally, the absorbance was measured at 510 nm (Genesys 10S UV-VIS, Thermo Scientific, USA) to determine the glucose concentration in the sample.

Statistical analysis

All experiments were performed in triplicates. Data were analyzed using the SPSS version 20 program (SPSS Inc., Chicago, I11., U.S.A.). The results are presented as mean ± standard deviation (SD), based on three independent replicates (n=3). One-way analysis of variance (ANOVA) was used to determine the differences in each parameter tested, followed by Duncan's test with α = 0.05. Principal component analysis (PCA) conducted to explore relationships among samples was performed by an in-house script using the MATLAB software (MATLAB V7.0, The Math Works Inc., Natick).

Results and Discussion

Kinetics of water absorption of germinated oats

To describe the kinetics of water absorption, the experimental data were fitted to four different models: Peleg's model, Weibull model, first-order kinetics, and exponential model (Table 1). According to Khazaei and Mohammadi (2009), the constant C2 in Peleg's model is inversely proportional to the water absorption capacity of

Table 1. Estimated kinetic parameters from four hydration models (Peleg, Weibull, first-order, and exponential) for ultrasound-treated and untreated oat seeds at different amplitudes (20%, 30%, 40%) and treatment durations (20, 30, 40 min).

Amplitude (%)	Ultrasound treatment time (min)	Peleg			Weibull			First-order		Exponential	
		C_1	C_2	U_{eq}	α	β	U_{eq}	k_1	U_{eq}	k_2	U_{eq}
0	0	0.13	0.87	1.34	0.36	9.80	1.49	0.62	0.89	0.41	0.82
20	20	0.05	1.75	2.25	0.44	10.00	1.68	0.47	1.02	0.45	0.90
	30	0.08	1.22	1.73	0.39	10.00	1.64	0.57	0.98	0.44	0.89
	40	0.06	1.69	2.19	0.44	10.00	1.77	0.47	1.07	0.51	0.94
30	20	0.06	1.37	1.93	0.36	10.00	1.67	0.66	0.99	0.44	0.92
	30	0.08	1.30	1.85	0.38	10.00	1.74	0.59	1.04	0.47	0.95
	40	0.06	1.60	2.11	0.43	10.00	1.75	0.49	1.06	0.50	0.94
40	20	0.13	0.81	1.40	0.30	10.00	1.63	0.84	0.96	0.39	0.92
	30	0.05	1.44	2.07	0.33	10.00	1.74	0.74	1.02	0.39	0.97
	40	0.11	0.92	1.53	0.31	10.00	1.69	0.78	1.00	0.40	0.95

*C_1: Peleg rate constant; C_2: Peleg capacity constant; U_{eq}: equilibrium moisture; α: shape parameter; β: scale parameters; k_1: first-order rate constant; k_2: exponential rate constant

food. For oat seeds, C2 increased with ultrasonic treatment, likely owing to physical changes in the seed structure induced by US during the initial hours of hydration. This structural alteration reduces the water absorption capacity, which is consistent with the behavior described by C_1. An increased US amplitude generally resulted in higher C_1 values, suggesting a faster initial absorption rate. This effect may be attributed to US disrupting the structure of the material and hindering the initial water penetration, as observed in studies on US-assisted drying (Garcia *et al.*, 2012). However, this trend was not consistent across all treatment times, and the C_2 values exhibited variability without a clear trend, possibly because of the competing effects of US on the material's microstructure and water-binding capacity (Jambrak *et al.*, 2010).

The Weibull model provided the best fit to the data, as evidenced by the high-fit statistics and well-defined shape (α) and scale (β) parameters, effectively capturing the progression of the absorption curve. The shape parameter (α<1) indicated a decreasing absorption rate over time, consistent with materials with porous structures (Kouhila *et al.*, 2001), meaning that $\alpha < 1$ means that the rate of absorption slows down over time. This behavior is typical in porous materials, where water initially enters quickly but slows as it diffuses deeper. Although all α values were less than 1, the variation among them indicates different water absorption dynamics. Lower α values represent a sharper initial absorption rate. This trend aligns with the porous structure of oats and the disruption caused by US treatment, which enhances water penetration.

The scale parameter (β) reflects the overall rate of water uptake, which varies with treatment conditions. The treated samples exhibited higher β values, indicating a longer time to reach equilibrium, whereas the untreated samples achieved equilibrium at 9.80.

Furthermore, the first-order and exponential models provided the rate constants (k_1 and k_2) for comparison, revealing fluctuations with US treatment, which further highlighted the complexity of the absorption process. Untreated samples had lower and higher k1 and k2 values than treated samples, emphasizing the need to consider each parameter individually.

Overall, the equilibrium moisture content of the US-treated samples was consistently higher than that of the untreated samples in all four models. These findings demonstrate a complex relationship between US treatment and moisture absorption. While US influences moisture absorption properties, its effects depend on both the amplitude and duration of the treatment. This aligns with previous research emphasizing the importance of optimizing US parameters for specific applications (Chemat *et al.*, 2011).

Table 2 describes the statistical indices evaluating the fit of hydration models under US treatment on the oat hydration kinetics using various US amplitudes and treatment times. Statistical analysis, including the R^2, SE, and mean relative error values, revealed that the Peleg and Weibull models demonstrated a good fit to the experimental data ($R^2 > 0.9$). Further analysis of these indices under different US conditions enabled the elucidation of US influence on oat hydration and determined the most appropriate model for predicting water absorption under specific processing parameters. This research provides valuable insights into optimizing oat processing

Table 2. Statistical evaluation of hydration model performance under different ultrasound conditions: coefficient of determination (R^2), standard error (SE), and mean relative error (P).

Amplitude (%)	Ultrasound treatment time (min)	Peleg			Weibull			First–order			Exponential		
		R^2	SE	P	R^2	SE	P	R^2	SE	P	R^2	SE	P
0	0	0.96	0.033	3.81	0.99	0.015	1.57	–0.06	0.158	15.36	0.84	0.061	7.42
20	20	0.95	0.040	4.49	0.99	0.013	1.57	0.07	0.181	16.31	0.90	0.060	6.19
	30	0.97	0.031	3.05	0.99	0.017	1.75	–0.06	0.177	16.25	0.89	0.056	6.08
	40	0.96	0.042	3.95	0.98	0.028	3.23	0.23	0.180	17.29	0.90	0.066	6.29
30	20	0.97	0.030	2.85	0.97	0.028	3.04	–0.24	0.188	17.60	0.91	0.050	4.48
	30	0.96	0.035	3.35	0.98	0.027	3.02	–0.05	0.187	16.91	0.89	0.060	5.56
	40	0.97	0.035	3.32	0.99	0.022	2.46	0.16	0.180	16.76	0.90	0.061	6.04
40	20	0.96	0.029	2.79	0.97	0.026	2.36	–0.81	0.192	15.83	0.86	0.053	5.60
	30	0.97	0.028	2.73	0.99	0.018	1.85	–0.83	0.213	17.29	0.92	0.043	3.79
	40	0.97	0.024	2.25	0.99	0.017	1.75	–0.77	0.199	15.83	0.89	0.050	4.77

*R^2: determination coefficient; P: mean relative error; SE: estimate standard error.

techniques for improved efficiency and product quality. Owing to the highest R^2 and lowest SE in Table 2, the Weibull model best describes the soaking kinetics of oat seeds across the investigated amplitudes and treatment durations. This finding was aligned with its effectiveness in modeling the soaking behavior of corn and soybean, as reported by Miranda *et al.* (2025).

The kinetics of water absorption in oats as a function of soaking time, expressed as the Peleg and Weibull models, is shown in Figure 1. Oat seeds exhibited consistent behavior across all groups with regard to the soaking kinetics. The hydration curves followed a characteristic pattern commonly observed in agricultural products, marked by an initial exponential increase in the water absorption rates. Over time, as the hydration progressed, and the grain moisture content gradually approached equilibrium (Ansari *et al.*, 2015; Vengaiah *et al.*, 2012). Untreated samples maintained a steady moisture content of approximately 0.9 g/g dry basis throughout the soaking process. In contrast, the ultrasonic-treated samples demonstrated significantly higher moisture content, ranging from 1.0 to 1.1 g/g dry basis. These results indicate that ultrasonic treatment effectively enhanced the water absorption capacity of the oat grains.

Effects of US treatment on germination rate of oat seeds

Visual comparison of germinated oat samples is shown in Figure 2, in which examples of oat at 30% amplitude and 40 min. ultrasonic time germinated for 30, 36, 42, and 48 h were given. It was found that 30 h indicates early germination, with limited root/shoot emergence. Moderate water uptake and germination progress can be seen at 36 h, whereas at 42 j and 46 h, more germination and advanced growth can be observed.

In Figure 3, US treatment improved germination, with rates increasing slowly at 30 and 36 h and then more rapidly at 42 and 48 h. At 48 h, 20% amplitude for 40 min achieved the highest germination rate (71.5% ± 9.1), which was statistically different from both untreated seeds (29.5% ± 6.3) and higher amplitude treatments. Duncan's multiple range test confirmed that treatments at 20% and 30% amplitudes for 30–40 min significantly enhanced germination compared to controls. The significantly lower germination rates ($P < 0.05$) at 40% amplitude (28.0% ± 2.8 at 48 h) highlight the detrimental effect of excessive sonication. The untreated oat seeds exhibited the lowest germination rates, ranging from 6.0% to 29.5%, whereas the oat seeds treated with 20% US amplitude achieved the highest germination rates, ranging from 33.0% to 71.5%. The germination rates of oat seeds treated with 30% and 40% US amplitude ranged from 19.5% to 60.5% and 12.5 to 46.5%, respectively. These effects are influenced by amplitude and exposure time, with moderate US intensity at 30% producing optimal cavitation without damaging seed structure.

The results of the germination rate experiment at 48 h revealed that the oat seeds treated with 20% US amplitude for 40 min exhibited the highest germination rate of 71.5%, followed by a germination rate of 69.5% at 20% US amplitude for 30 min. The increase in germination rate because of US stimulation prior to soaking was closely related to enhanced oxygen content and water absorption. US alters the permeability and deformation of the cell membrane through the effects of cavitation and microcurrents. This alteration was evident in

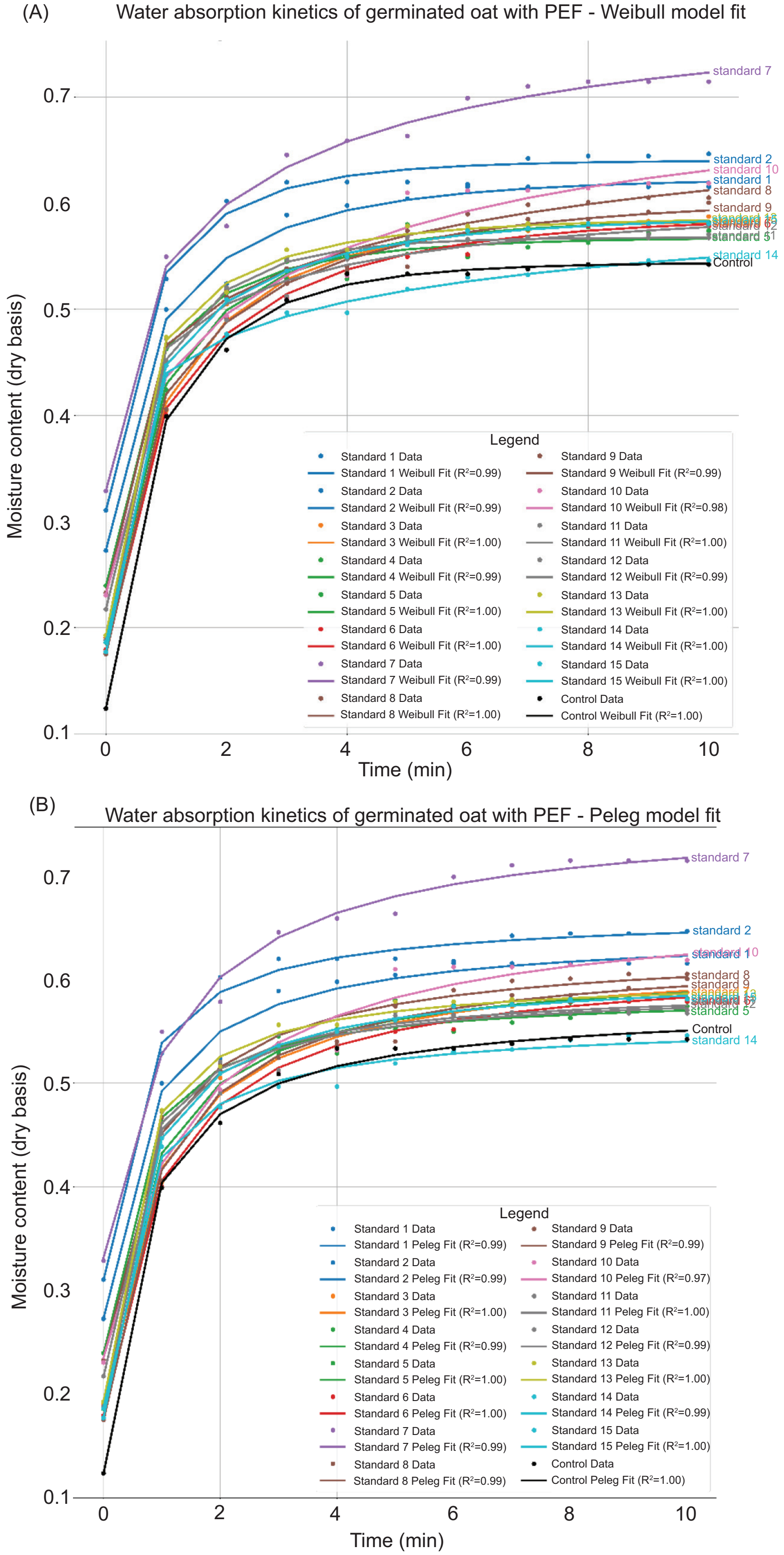

Figure 1. Hydration kinetics of ultrasound (US)-treated and untreated oat seeds as modeled by (A) Weibull and (B) Peleg models. Data reflect moisture absorption over 10 h. US amplitudes of 20%, 30%, and 40% were applied at varying durations. Curves illustrate model fit and the effect of US on hydration behavior.

Figure 2. Visual comparison of germinated oat seeds after 30, 36, 42, and 48 h. Samples were treated with US at 30% amplitude for 40 min.

the presence of holes in the seed coat after sonication, which facilitated mass transport (e.g., water, nutrients, and growth regulators). Petru *et al.* (2018) also observed that ultrasonic cavitation induces micro-erosions on seed coats, increasing permeability and promoting mass transfer, which is crucial for seed hydration and germination. In addition, higher amplitude can increase cavitation intensity. Elevated US amplitude leads to more intense cavitation, resulting in stronger shear forces and microjets that can disrupt seed coats, enhancing water uptake and seed permeability. This disruption facilitates earlier and more uniform germination. These changes promote metabolism by activating enzymes, accelerating nutrient synthesis, and triggering other physiological effects that enhance germination (Xia *et al.*, 2020).

The observed enhancements in hydration and germination parameters can be attributed to the mechanical effects of acoustic cavitation generated during US treatment. Cavitation causes the formation and collapse of microbubbles, which create localized high-pressure and shear forces that disrupt the seed coat, increase pore size, and enhance mass transfer (Chemat *et al.*, 2011; Petru *et al.*, 2018). These physical alterations facilitate faster water penetration, as evident from the higher water absorption rates and equilibrium moisture contents in ultrasonicated samples (Table 1). Moreover, cavitation-induced microchannels likely promote oxygen diffusion and enzymatic activation, which are critical for the initiation of germination. This explains the significantly improved germination rates and root elongation observed, particularly under 20–30% amplitude and 30–40 min sonication. However, excessive cavitation at 40% amplitude may cause structural damage or oxidative stress, leading to reduced germination efficiency and antioxidant responses. These findings support previous reports that controlled cavitation intensity enhances seed metabolism and viability while preserving cellular integrity (Gong *et al.*, 2024; Xia *et al.*, 2020).

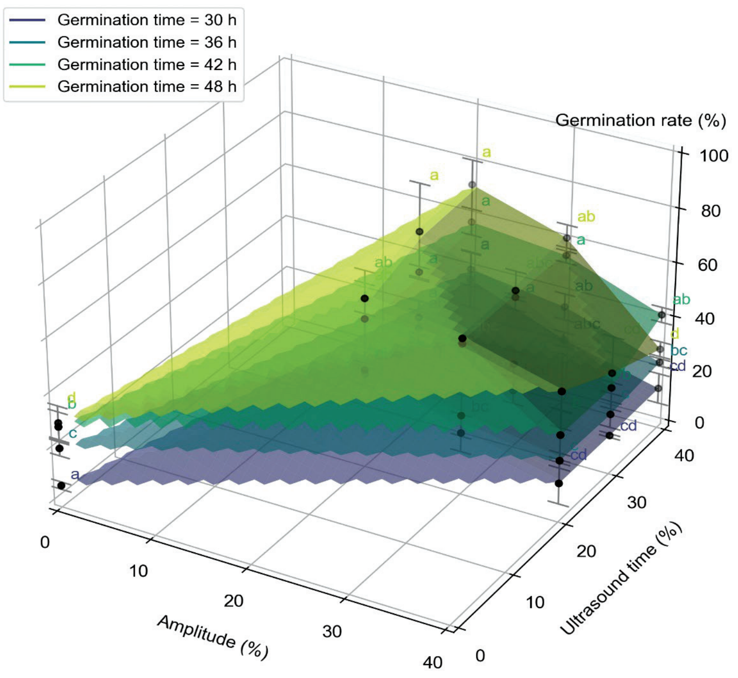

Figure 3. Germination rates (%) of ultrasound-treated and untreated oat seeds measured at 30, 36, 42, and 48 h of germination. Treatments vary by amplitude (20%, 30%, and 40%) and duration (20, 30, and 40 min). Data are presented as mean ± SD. Different superscript letters within the same germination time indicate significant differences among treatments (P < 0.05, DMRT).

Effects of US treatment on root length of germinated oats

Figure 4 shows the root lengths of germinated oats treated with US at different amplitudes and durations. The germination times (30, 36, 42, and 48 h) of the US-treated and untreated oat seeds showed similar trends. However, US treatment significantly enhanced root length development. US significantly affected root elongation ($P < 0.05$), particularly after 48 h of germination. The longest root lengths (>5 mm) were observed in treatments with 20% amplitude for 40 min and 30% amplitude for 40 min, and these values were significantly higher than those from untreated seeds. The root length improvements correlated with enhanced water uptake and enzyme activity, suggesting that optimal US conditions activate physiological processes supporting root growth.

US treatment improved root length and moisture content compared with untreated oat seeds, demonstrating its effectiveness in promoting germination and hydration. By enhancing the efficiency of water absorption, improving cell division, and activating key enzymes, US positively affects oat seed germination and root development, leading to faster germination and improved root growth (Ramteke *et al.,* 2015). Longer exposure time can prolong cavitation effects. Extended US exposure allows sustained cavitation effects, leading to deeper penetration of water into seeds and prolonged activation of hydrolytic enzymes. This supports enhanced mobilization of stored nutrients and stimulates metabolic activity, promoting seed germination. Gong *et al.* (2024) demonstrated that variable frequency ultrasonic treatment significantly increased the percentage of maize seed germination and radicle length, indicating that prolonged exposure enhances germination outcomes. However, determining the ideal root length for maximum bioactivity in germinated oats is complex and varies based on the specific bioactive compounds of interest and experimental conditions. Hu *et al.* (2025) investigated the effects of germination on steroidal saponin levels in oats. The research found that germination at 20°C for 7 days significantly increased the total steroidal saponin content to 3265.54 µg/g, doubling its original concentration. This suggests that extended germination periods may enhance bioactive compound levels, potentially correlating with root development. Moisture content and soaking time significantly influenced germination, with US-treated oat seeds exhibiting superior hydration and root length than untreated oat seeds. These findings are consistent with those of previous studies on various plants, confirming the beneficial effects of US on germination and seedling growth (Liu *et al.*, 2016). This study highlights US as a promising nonthermal technology with significant potential for enhancing seed germination and hydration processes in the agricultural industry.

Effect of US treatment on the TPC of germinated oats

US treatment enhanced the TPC of germinated oats, with untreated germinated oats exhibiting the lowest content (Figure 5). The phenolic content increased with longer germination times, supporting the hypothesis that sprouting boosts antioxidant levels. US amplitude significantly affected phenolic content, particularly at 20% and 30% amplitudes, with US20-T40 at 42 h yielding the highest TPC ($P < 0.05$). Post-hoc analysis indicated that excessive amplitude (40%) led to statistically lower TPC values, likely because of phenolic degradation.

Moderate US (20–30%) promotes phenolic biosynthesis and enhances extraction of bound phenolics, resulting in increased TPC. However, excessive US (40%) may degrade phenolic compounds or alter metabolic balance, leading to reduced antioxidant levels. Similarly, DPPH activity is influenced by both TPC and other antioxidant compounds activated during germination. Statistical analysis confirmed significant differences in phenolic

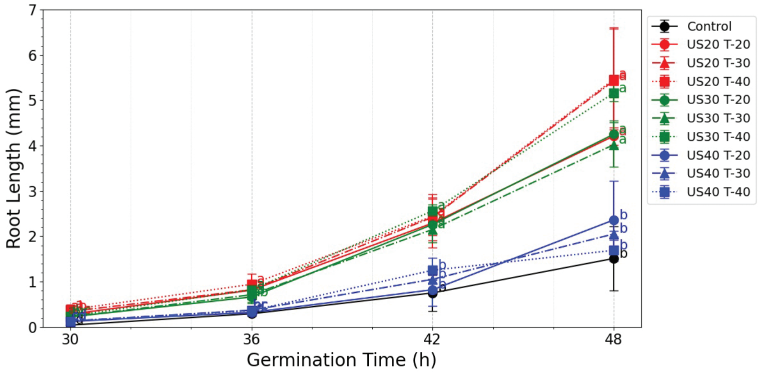

Figure 4. Root length (mm) of germinated oats as affected by ultrasound treatment at different amplitudes (US20, US30, and US40) and treatment durations (T20, T30, and T40). Data represent mean ± SD. Different superscript letters at each time point (30–48 h) indicate significant differences between treatments ($P < 0.05$, DMRT).

content based on the US amplitude and treatment time. Phenolic compounds, which are key contributors to the antioxidant activity of cereals, increase significantly during soaking and sprouting (Kruma *et al.*, 2016). These secondary metabolites provide numerous positive physiological effects, highlighting the importance of sprouting for enhancing the nutritional and functional value of grains (Peng *et al.,* 2015).

The observed increase in TPC with moderate US (20–30% amplitude) can be attributed to enhanced mass transfer and cell wall disruption caused by cavitation. These mechanical effects improve the release of bound phenolic compounds from the seed matrix, increasing their extractability (Chemat *et al.*, 2011). In addition, US may activate phenylpropanoid pathway enzymes such as phenylalanine ammonia lyase (PAL), which catalyzes de novo phenolic biosynthesis during early germination (Kruma *et al.*, 2016). This dual mechanism—physical liberation of existing phenolics and biosynthetic activation—explains the significantly higher TPC at moderate amplitudes, particularly at 42 h of germination.

Effect of US treatment on 1,1 diphenyl-2-picrylhydraziyl (DPPH) radical scavenging activity of germinated oats

This study demonstrated that germination after 48 h resulted in the highest DPPH radical scavenging activity in oat seeds (Figure 6). DPPH activity was significantly influenced by both germination time and US conditions (P < 0.05). The highest antioxidant activity was recorded at US30-T40 at 42 h, which was statistically higher than both control and other treatments. The observed variation in DPPH activity, despite high TPC in some groups, underscores that antioxidant activity is multifactorial and not solely dependent on phenolic content. Short sonication at low amplitudes (US20-T20) significantly underperformed compared to the control (P < 0.05), emphasizing the need for adequate US exposure to stimulate antioxidant pathways. However, it was noticed that while it is expected that antioxidant activity should increase with germination duration, the lower DPPH value for US20-T20 at 42 h compared to the control may reflect a combined influence of suboptimal US stimulation and delayed antioxidant biosynthesis. At low amplitude and short exposure (US20-T20), the cavitation effect may be insufficient to activate metabolic pathways responsible for antioxidant compound synthesis (e.g., phenolics, flavonoids). However, at 42 h, the depletion of initial antioxidant precursors or mild oxidative imbalance may further reduce DPPH activity. This specific combination of mild pretreatment and prolonged germination may not provide a sufficient stress-induced or enzymatic trigger to maximize antioxidant pathways.

These results were consistent with the observed trends in germination time and root length, which aligned with the study by Van Huang *et al.* (2011), who reported an increase in antioxidant activity and chemical changes during wheat germination. Similarly, Naumenko *et al.* (2022) observed the highest DPPH radical scavenging activity in hull-less barley. It can be concluded that US treatment of oat seeds before germination positively influenced DPPH radical scavenging activity, potentially by enhancing the metabolic processes associated with germination. Further research is needed to elucidate the specific mechanisms underlying this effect and to explore the potential of US for improving the nutritional quality of oat seeds and other grains. Research published by Kim *et al.* (2013) examined the changes in antioxidant and antiproliferation activities of germinated winter cereal crops, including oats, and found that the highest total polyphenol content was observed

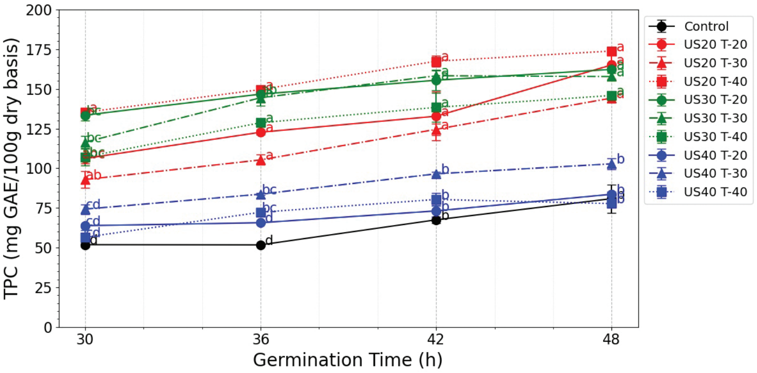

Figure 5. **Total phenolic content (TPC, mg GAE/100 g DW) of germinated oats after US treatment at various amplitudes and durations (US20, US30, and US40: US treatment at 20%, 30%, and 40% amplitude; T20, T30, T40: Treatment duration of 20, 30, and 40 min). Data show mean ± S.D. different letters in the same germination time indicate differences between the treatments by DMRT (P < 0.05).**

post-germination, indicating an increase in antioxidant activity. This could because of the fact that US generates acoustic cavitation, which can disrupt cell walls, increase mass transfer, and activate metabolic pathways. In the context of germinated oat seeds, these effects may enhance the release or synthesis of bioactive compounds (e.g., avenanthramides, phenolics, and saponins), which in turn increases the antioxidant activity. From this study, it was observed that TPC (Figure 5) is highest at US20-T40 at 42 h, whereas the DPPH scavenging activity is highest at US30-T40 at 42 h. This difference suggests that while TPC correlates with antioxidant capacity, other compounds such as flavonoids, vitamins, or enzyme activity may contribute more significantly to DPPH scavenging at certain US conditions, particularly under moderate sonication (30%). It was also observed that DPPH value at 42 h for US20-T20 decrease compared to the control sample. This might be because of shorter sonication at low amplitude may not sufficiently enhance antioxidant synthesis or release bound phenolics. In addition, mild US might induce slight oxidative stress without sufficient protective response, lowering net antioxidant capacity.

In contrast to results observed for TPC, at high amplitudes (e.g., 40%), cavitation becomes more intense and may result in localized oxidative stress or thermal hotspots, which could degrade phenolic structures or suppress enzymatic pathways, thereby reducing TPC and antioxidant activity (Naumenko *et al.*, 2022). This is supported by the comparatively lower DPPH values at 40% amplitude despite extended germination. The divergence between TPC and DPPH trends also indicates that non-phenolic antioxidants (e.g., flavonoids, vitamin C, and enzyme activity) may contribute to DPPH scavenging under some conditions, particularly at US30-T40, which showed the highest antioxidant activity.

Effect of US treatment on β-glucan content of germinated oats

β-glucan is a dietary fiber commonly found in grains, with barley and oat seeds being particularly rich sources. Although no statistically significant differences ($P > 0.05$) were observed between US-treated and untreated oats in β-glucan levels, germination time had a significant effect ($P < 0.05$) on β-glucan degradation. β-glucan content decreased progressively with longer germination, supporting the hypothesis that enzymatic breakdown via β-glucanase activity is time-dependent rather than treatment-dependent (Figure 7).

Our findings align with previous research, such as the study by Doehlert and McMullen (2003) on wheat, rye, triticale, and barley, which showed a decrease in β-glucan concentration and integrity upon germination. Similarly, research by Hübner and Arendt (2013) on whole grains demonstrated that β-glucans break down, leading to decreased β-glucan content as the grain germinates, possibly because of enzyme activity during germination. During germination, the seed begins to sprout, and this process involves the activation of various enzymes, including β-glucanase, which breaks down soluble β-glucans. As germination time increases, the activity of β-glucanase and other enzymes responsible for breaking down cell wall components also increases. These enzymes break down complex carbohydrates, including β-glucans, into simpler sugars that seedlings can use as an energy source for growth. The resulting breakdown of β-glucan during germination leads to a reduction in the overall β-glucan content of the oat seeds. It is important to note that although sprouting can decrease β-glucan content, it can increase the content of other beneficial compounds, such as certain vitamins, minerals, and antioxidants. In addition, the decrease in β-glucan content during

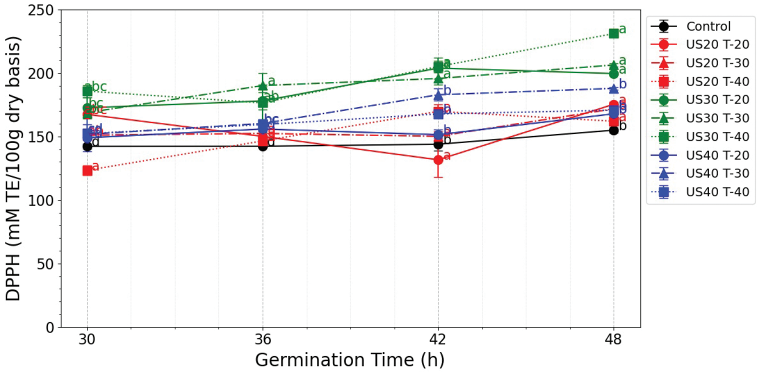

Figure 6. DPPH radical scavenging activity (mg TE/100 g DW) of germinated oats treated with US at different amplitudes and durations. (US20, US30, and US40: US treatment at 20%, 30%, and 40% amplitude; T20, T30, and T40: Treatment duration of 20, 30, and 40 min). Data show mean ± S.D. Different letters (a–h) in the same germination time indicate differences between the treatments by DMRT ($P < 0.05$).

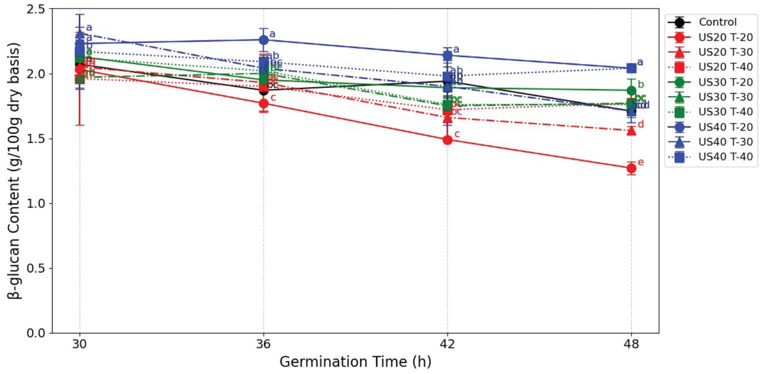

Figure 7. β-glucan content (%, dry basis) of germinated oats after ultrasound (US) treatment at different amplitudes and durations evaluated at 30, 36, 42, and 48 h of germination (US20, US30, US40: US treatment at 20%, 30%, and 40% amplitude; T20, T30, T40: Treatment duration of 20, 30, and 40 min). Bars represent mean ± SD. Different superscript letters within the same time point indicate statistically significant differences (P < 0.05).

germination can be influenced by factors such as temperature, humidity, and the specific type of oat used. The levels of β-glucan decreased progressively with germination time because of the activation of β-glucanase, which breaks down cell wall polysaccharides to support seedling growth. US treatment did not significantly alter the levels of β-glucan, likely because the enzymatic degradation was more dependent on germination duration than on pre-treatment intensity.

In conclusion, US treatment of oat samples before germination positively impacts phenolic content, DPPH radical scavenging activity, and β-glucan content through mechanisms involving the release of bound compounds, activation of biosynthetic pathways, improved bioavailability, and mechanical disruption to enhance extraction. These effects collectively contribute to the higher levels of bioactive compounds in the treated samples, making them potentially more beneficial for human health.

PCA score plot is described in Figure 8(A). This statistical tool was used to explore the relationships among oat samples subjected to different US treatments. Each point represents a sample, with PC1 and PC2 on the axes corresponding to the two principal components that explain the largest portions of variation in the data: 67.58% and 17.07%, respectively. These principal components accounted for 84.65% of the total variation in data. The remaining 15.35% of variation is attributed to less significant variables and noise. The samples cluster distinctly by treatment group, with untreated (black) and US-treated samples (blue: 20%, red: 30%, and green: 40%) forming visually separable groupings. This indicates that US amplitude has a substantial influence on sample variation. PCA validation (e.g., KMO test, scree plot) was not performed in this study but is recommended for future research to confirm factor reliability and data dimensionality.

The clustering of samples into four distinct groups suggests varying responses to US treatment. Figure 8(B) shows a PCA loading plot that highlights the correlations between each variable and the principal components. The plot includes five variables related to oat properties: root length, germination rate, DPPH scavenging activity, β-glucan content, and TPC. The loading directions and lengths reflect the variables' contributions to PC1 and PC2. Closely grouped loading plots, such as the red sample (US 30%), show DPPH and TPC, indicating a strong positive correlation, while β-glucan content shows a negative correlation with PC1. This loading plot provides an insight into the variables driving the observed variations in oat characteristics, assisting in interpreting the relationships uncovered by the PCA.

Conclusions

This study demonstrated the significant benefits of US technology in enhancing oat seed germination and quality. US treatment improves water absorption, leading to increased germination rates, root length, TPC, and DPPH radical scavenging activity. These findings underscore the potential of US to positively influence the water absorption, germination, and antioxidant capacity of oat seeds. Based on the findings of this study, optimal US treatment conditions varied depending on the desired outcome.

However, the results generally suggested that the optimal conditions for producing germinated oats with high phenolic content, antioxidant activity, and acceptable β-glucan levels are 30% US amplitude, 40 min of sonication, and 42 h of germination. These parameters balance hydration efficiency, antioxidant potential, and compositional integrity. Even though at 48 h with 20% amplitude

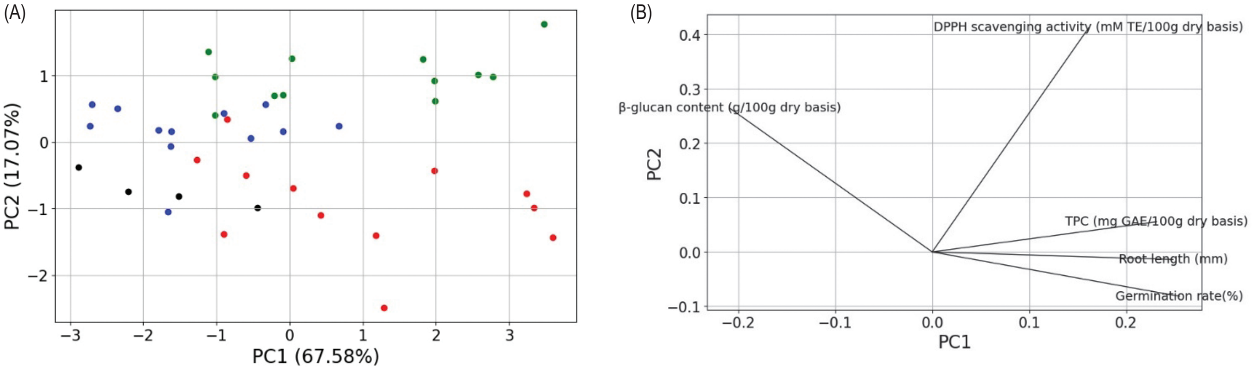

Figure 8. Principal component analysis (PCA) of oat seed characteristics following ultrasound treatment. (A) Score plot showing sample clustering by amplitude (20%, 30%, and 40%) and control; (B) Loading plot indicating contribution of key variables (TPC, DPPH, β-glucan, germination rate, and root length) to PC1 and PC2.

yielded the highest germination rate, 42 h under 30% amplitude showed the best combination of germination rate, phenolic content, and antioxidant capacity, making it the optimal overall condition. The improved nutritional and functional qualities of seeds germinated using US-highlight their potential for industrial applications in the development of functional foods, nutraceuticals, and fortified grain-based products. The improved nutritional and functional qualities of oat seeds germinated using US highlight their application in functional foods, health supplements, and plant-based product formulations

While this study confirms the potential of US-assisted germination to enhance the nutritional and functional properties of oat seeds, it does not assess the sensory quality or flavor profile of the treated grains. Prolonged sonication or high amplitudes may potentially alter taste, texture, or aroma—factors that are critical for consumer acceptance and product development. Therefore, future studies should incorporate sensory evaluation to determine the palatability and market viability of oats germinated using US in food applications.

In addition, although the US parameters tested in this study were successful at the laboratory scale, scalability and energy efficiency remain important considerations for industrial application. High-power US systems capable of continuous processing must be evaluated in terms of processing throughput, energy cost, and equipment integration within existing germination lines. Research into pilot-scale implementation and economic analysis would provide valuable insight into commercial feasibility for food and nutraceutical industries.

Author Contributions

Conceptualization was done by Saowaluk Rungchang; methodology was looked into by Sudarat Jiamyangyuen and Saowaluk Rungchang; investigation was done by Tien Mai Thi Cam, Malee Pisitchaiwet, Nonnaphat Natpanyaporn, Tida Jaopitakwong, Nguyen Thi Khanh Hoa, and Chayanid Sringarm; data curation was the responsibility of Saowaluk Rungchang, Srivikorn Ditudompo, and Thiranan Kunanopparat; writing—original draft preparation was done by Chayanid Sringarm; writing—reviewing and editing were done by Sudarat Jiamyangyuen, Saowaluk Rungchang, Sakunna Wongsaipun, Srivikorn Ditudompo, and Thiranan Kunanopparat; funding acquisition was the concern of Sudarat Jiamyangyuen and Srisuwan Naruenartwongsakul; validation and resources were taken care of by Saowaluk Rungchang, Sakunna Wongsaipun, Srivikorn Ditudompo, and Thiranan Kunanopparat; supervision and project administration were looked into by Sudarat Jiamyangyuen and Saowaluk Rungchang. All authors reviewed and read the manuscript, and agreed upon the published version of the manuscript.

Conflicts of Interest

The authors declared no conflicts of interest.

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