ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

Ultrasound-Assisted Germination of Buckwheat: Impact on Anti-nutritional and Antioxidant Content*

Karabuğdayın Ultrases-Destekli Çimlendirilmesi: Anti-Besinsel ve Antioksidan Bileşenler Üzerine Etkisi

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Abstract

Buckwheat is a highly nutritious grain that contains macro and micronutrients with antioxidant potential. However, some anti-nutritional components in buckwheat, such as phytic acid and condensed tannins, reduce its bioavailability. Germination is an ancient and natural method to improve the nutritional value of cereal grains. Controlled germination processes increase the nutritional value of pseudocereals by reducing the amount of antinutrients. Thus, the bioavailability of nutrients improved. Ultrasound-assisted germination can be a good alternative for increasing nutritional and bioactive values and mitigating the anti-nutritional properties of buckwheat by accelerating enzyme activity and biochemical changes by affecting the cell membrane structure. This study investigated the changes in chemical composition, phenolic content, antioxidant activity, color, and anti-nutrient content of buckwheat grains (*Fagopyrum esculentum* Moench. cv. Aktaş) germinated for 72 hours by traditional and ultrasound-assisted methods. The highest total phenolic content (498.57$\pm$2.93 mg GAE 100 g^{-1}) and antioxidant activity (10.91$\pm$1.62 μmol TEAC g^{-1}) of buckwheat were measured in the germinated sample using the ultrasound-assisted method. However, the color changes were not notable during 72 h of germination. The lipid (3.06 – 3.39%) and protein contents (12.56 – 15.05%) were increased after germination. The starch content was 58.58% in ungerminated buckwheat and decreased (52.36%) after 72 h of germination using the ultrasound-assisted method. In germinated samples, tannin content was reduced by 43 – 54%, whereas a slight reduction was observed in phytic acid concentrations. Ultrasound-assisted germination can be a valuable alternative to improve the chemical composition and bioactive potential and mitigate the anti-nutritional properties of buckwheat grains. Germinated grain, flour, or processed products might be good alternatives to traditional cereal consumption because of their improved functional properties.

Keywords: Aktaş, Total phenolic content, Antioxidant activity, Tannin, Sprouting, Ultrasound

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Öz

Karabuğday antioksidan potansiyele sahip makro ve mikro besin elementleri açısından zengin bir pseudo-tahıldır. Fakat bileşiminde bulunan fitik asit ve kondanse tanenler gibi bazı anti-besinsel bileşenler karabuğdayın biyoyararlanımını azaltmaktadır. Çimlendirme tahıl tanelerinin besin değerinin artırılmasında eski ve doğal yollardan biridir. Kontrollü çimlendirme prosesleri, anti-besinsel bileşen konsantrasyonunu azaltarak pseudo-tahılların besin değerini artırmakta ve böylece besinlerin biyoyararlılığını iyileştirmektedir. Ultrases dalgaları enzim aktivitesini hızlandırırken hücre membran yapısını da etkileyerek kimyasal değişimlere neden olmaktadır. Bu nedenle, ultrases destekli çimlendirme işlemi besinsel ve biyoaktif özelliklerin artırılması ve anti-besinsel özelliklerin azaltılması için iyi bir alternatif olabilir. Bu çalışmada, karabuğday (*Fagopyrum esculentum* Moench. var. Aktaş) geleneksel ve ultrases destekli yöntemlerle 72 saat süresince çimlendirilmiştir. Çimlendirme süresince kimyasal kompozisyondaki değişimin yanı sıra anti-besinsel bileşenler, toplam fenolik madde miktarı, antioksidan aktivite ve renk değerlerindeki değişim de incelenmiştir. Çimlendirme işlemi sonrasında en yüksek toplam fenolik madde miktarı (498.57 ± 2.93 mg GAE 100 g^{-1}) ve antioksidan aktivite (10.91 ± 1.62 μmol TEAC g^{-1}) ultrases destekli çimlendirilen karabuğday örneklerinde ölçülmüştür. Buna karşın 72 saatlik çimlendirme sürecinde renk değerlerinde belirgin bir değişim gözlenmemiştir. Karabuğday tanesinde çimlendirme sonucunda yağ (%3.06 – 3.39) ve protein (%12.56 – 15.05) konsantrasyonu artmıştır. Çimlendirilmemiş karabuğdayda nişasta konsantrasyonu %58.58 olarak belirlenirken ultrases destekli çimlendirme yöntemi kullanılan örneklerde 72 saat sonunda nişasta konsantrasyonu (%52.36) azalmıştır. Tanen miktarı ise %43 – 54 düzeyinde azalırken fitik asit miktarında daha sınırlı bir azalış olduğu belirlenmiştir. Ultrases destekli çimlendirme yönteminin, tahıl tanesinin kimyasal kompozisyonunun iyileştirilmesi, biyoaktif potansiyelin artırılması ve anti-besinsel özelliklerin azaltmasında faydalı bir yöntem olabileceği belirlenmiştir. Çimlendirilmiş tane, taneden elde edilen un veya diğer işlenmiş ürünler, çimlendirme ile artan fonksiyonel özellikleri nedeniyle tahılların geleneksel tüketimine daha iyi bir alternatif olabilirler.

Anahtar Kelimeler: Aktaş, Toplam fenolik madde miktarı, Antioksidan aktivite, Tanen, Çimlendirme, Ultrases

1. Introduction

Buckwheat, a pseudo-cereal from the Polygonaceae family, is primarily cultivated in common (*Fagopyrum esculentum* Moenchand) and tartary (*Fagopyrum tataricum L.* Gaertn) buckwheats (Békés et al., 2017; Tömösközi and Langó, 2017). Buckwheat is an annual plant that can be adapted to a variety of agricultural systems worldwide, and is produced mainly in Europe (56%), Asia (34%), and the USA (9%) (Anonymous, 2022a). Buckwheat breeding is increasing in Türkiye, and Aktaş has been the most recent and cultivated variety by the Bahri Dagdas International Agricultural Research Institute (Anonymous, 2022b). Buckwheat has gained interest due to its nutritious and gluten-free nature, although pseudocereals are generally less widely consumed than common cereals such as maize, rice, and wheat (Agregán et al., 2023; Niro et al., 2019).

Buckwheat is a good source of nutritional and anti-nutritional content (Altıkardeş and Güzel, 2024). The high protein (13%) content is primarily composed of globulin, and the essential amino acid profile is the notable characteristic of buckwheat (Agregán et al., 2023; Dizlek et al., 2009). The sulfur-containing amino acid contents of buckwheat, methionine ($0.1 – 2.3$ g 100 g^{-1} protein), and cysteine ($2.06 – 3.27$ g 100 g^{-1} protein) are comparable to or higher than those of rice and maize, as well as leucine, phenylalanine, lysine, and threonine (Tömösközi and Langó, 2017). The starch content varied between 60 and 70%, mainly amylose ($25 – 50\%$) in buckwheat grains, and the amylose content was higher than that of many common cereals (Berghofer and Schoenlechner, 2002). Lipids ($2 – 4\%$) are primarily concentrated in the embryo of buckwheat grain and provide many unsaturated fatty acids, such as oleic, linoleic, and palmitic acids ($75 – 80\%$ of total lipids) (Kokten, 2023; Tömösközi and Langó, 2017). Many essential micronutrients in buckwheat, including iron, magnesium, phosphorus, copper, manganese, and vitamins B and E, are particularly noteworthy (Alvarez-Jubete et al., 2009; Bhinder et al., 2022). Furthermore, buckwheat grains are rich in phytic acid and tannins (anti-nutrients) (Altıkardeş and Güzel, 2024; Zhang et al., 2015). However, excessive levels of antinutrients may reduce the grain's nutritional bioavailability (Zhang et al., 2012).

Germination is a natural and effective way to improve the nutritional bioavailability and nutraceutical quality of whole grains (Agregán et al., 2023). During soaking and germination, the phytic acid content decreased with increasing phytase activity. Thus, the bioavailability of many mineral compounds can be increased (Xu et al., 2018). Most research has been focused on the traditional germination of pseudocereals, which involves soaking the grains in water for a certain period (Paucar-Menacho et al., 2017; Shreeja et al., 2021; Thakur et al., 2021). However, traditional germination methods have challenges such as breaking the hard grain coating, low awakening rate of the seeds, and deterioration due to prolonged water soaking. Various pre-treatment methods have been investigated recently such as ultrasound and alkali assisted germination, and each might impact the grain's nutritional values (Altıkardeş and Güzel, 2024; Díaz González et al., 2019; Ding et al., 2018). Ultrasound treatment is a novel approach that stimulates seed accumulation of health promoting compounds (Ding et al., 2018). This study represents the first comprehensive evaluation of the chemical compositional changes that occur during the germination of Aktaş, a widely cultivated variety of buckwheat.

The objective of the current study was to comprehensively assess the effects of two germination techniques (ultrasound-assisted and traditional) and different germination times (0, 12, 24, 48, and 72 h) on the nutrient, anti-nutrient, and antioxidant activities of the Aktaş variety buckwheat.

2. Materials and Methods

2.1. Material

Buckwheat (*Fagopyrum esculentum* Moench. cv. Aktaş), a registered variety, was obtained from the Bahri Dagdas International Agricultural Research Institute, Konya, Türkiye. The phenolic standards were purchased from Sigma-Aldrich. (St. Louis, Mo) and all other analytical-grade chemicals were purchased from Merck (Darmstadt, Germany).

2.2. Germination

Buckwheat grains were weighed after being cleaned of impurities. 100 g of grains were washed and drained. Traditional soaking was done with 1:3 (w/v) grain/water concentration for 12 h at 25±2°C. Ultrasonic power (Sonorex, Bandelin, Germany) was applied at 37 kHz with 100% amplitude for 30 min with the same grain/water concentration at 25±2°C for ultrasound-assisted germination of the whole buckwheat grain. Treated grains by the traditional and

ultrasound-assisted methods were germinated in a quartz jar under dark conditions and incubated for 12, 24, 48, and 72 h at 25±2°C (Memmert, Germany). Ungerminated grains were used as control samples. All samples were prepared in two independent replicates for each treatment and were dried at 48±2°C for 40 h, followed by ground to 500 μm. The germination percentage of the Aktaş grain (GP%) was calculated according to the following equation (Eq.1) by Beyaz (2023).

$$Germination\ Percentage\ (\%) = \frac{Germinated\ grain}{Whole\ grain} x\ 100 \qquad (Eq.1).$$

2.3. Proximate composition

The moisture content of the control and germinated samples was determined using a moisture analyzer (Pfeuffer GMBH, Germany). Crude lipid, starch, protein, and ash contents were determined using NIR spectroscopy (Perten, PerkinElmer Inc., ABD). As previously described by Peiris et al. (2019), the spectroscopic measurements were done at 950 – 1650 nm.

2.4. Color changes

The Lightness (L*), redness (+a*), yellowness (+b*), Chroma (C*), and hue angle (h°) color coordinates were determined using a colorimeter (Minolta CM-3600d, Japan) (Güzel et al., 2022). The total color change (ΔE) was calculated according to the following equation (Eq.2) described previously by Turksoy et al. (2024).

$$\Delta E = \sqrt{(\Delta a^2 + \Delta b^2 + \Delta L^2)} \qquad (Eq.2).$$

2.5. Total phenolic

The Folin-Ciocalteu spectrophotometric method was performed with minor modifications to measure the total polyphenols (Paucar-Menacho et al., 2017). The phenolic content (TPC) was extracted from the germinated and control samples using ultrasonic power (37 kHz, 100% amplitude) for 30 min at 25±2°C. The extraction solvent was acidified water (0.5%, v/v) and ethanol at a 20:80 (v/v) ratio. 1 g of sample flour was extracted with 10 mL of extraction solvent and centrifuged (Sigma 3-30K, Germany) at 8000 x g at 4°C for 10 min. 500 μL of the extracted sample was mixed with 0.2 N of Folin reagent. 1 mL of Na_2CO_3 was added to the solution and mixed well. After that, the entire mixture was left in the dark for an hour at room temperature. The Shimadzu spectrophotometer (UV-1800, Japan) was used to measure the absorbance values at 720 nm. The total phenolic content was reported as milligrams of Gallic Acid Equivalent (GAE) per 100 grams of dry weight.

2.6. Tannin

The concentration of tannin was determined using the colorimetric vanillin method (Güzel, 2021). The sample extract was mixed with sulfuric acid (70%, w/w) and vanillin solution (1%, w/v), and color changes were recorded at 500 nm. Catechin standard equation was used for the tannin quantification, and the results were given as mg CE (Catechin Equivalent) per g dry weight.

2.7. Phytic acid

Phytic acid in the germinated and control samples was extracted with HCl (0.2 N), as described by Haug and Lantzsch (1983). Fe III solution was mixed with extracts and centrifuged at 5000 x g for 10 min. The measurements were done using a Shimadzu spectrophotometer at 519 nm. A phytic acid standard solution was used as an external standard to calculate the phytic acid in samples, and the results were given as mg 100 g^{-1} dry weight.

2.8. Radical scavenging activity (DPPH)

1900 μL of 0.1 mM DPPH solution was mixed with the 50 μL of sample extract prepared to determine the phenolic content, and the mixture was left at RT for 30 min (Brand-Williams et al., 1995). Following the incubation, the mixture absorbance was recorded at 515 nm. The results were given as μmol TEAC (Trolox Equivalent Antioxidant Activity) g^{-1}.

2.9. Statistical Analysis

The GLM procedure was used to analyze the results (Minitab, LLC). All measurements were done in triplicate.

Tukey's test was performed to analyze the statistical difference between mean values at the $p < 0.05$ significance.

3. Results and Discussion

3.1. Germination percentage and proximate composition

The germination percentage of Aktaş buckwheat increased significantly during germination and was 98.5% at 72 h of germination (*Figure 1*). Germination index can be varied among varieties (Altuner et al., 2022). The average protein content in the control samples was 12%. These results agree with the declaration (11 – 14%) of the Bahri Dagdas International Agricultural Research Institute. The starch, lipid, and ash content of ungerminated Aktaş buckwheat was 58.6%, 2.7%, and 1.1%, respectively. Kumari et al. (2023) determined similar protein (13%) and lipid (2.7%) content in ungerminated common buckwheat. The changes in proximate composition during germination are shown in *Figure 2*. After 72 h of germination, ash and lipid content increased ($p < 0.05$) and varied between 1.8 – 2.3% and 3.0 – 3.4%, respectively. Protein contents in Aktaş buckwheat increased slightly (12.3 – 12.6%), whereas starch content decreased (52%) only after ultrasound assisted germination ($p < 0.05$). The alteration in starch molecule during the germination may have rendered it more vulnerable to hydrolysis. Thakur et al. (2021) found an increase (19.7%) in protein content after germination. Similarly, the protein content in common buckwheat increased after germination (Kumari et al., 2023). Zhang et al. (2015) claimed that several biochemical processes during germination activated several enzymes involved in protein synthesis. Thus, some proteins can be synthesized by the biochemical reaction, while others hydrolyzed by protease.
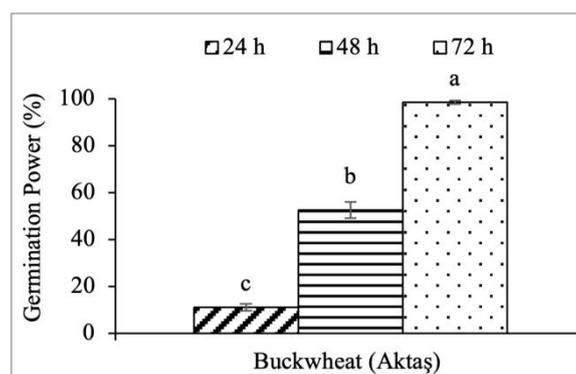

Figure 1. Germination percentage of buckwheat

3.2. Color changes

During the germination of Aktaş buckwheat, the changes in *L**, *a**, *b**, C, and *ℎ* color values were measured (*Table 1*). *L** (lightness) values expressed black-to-white color at 0 to 100 and was 86.9 in raw buckwheat. The redness (*a**) values increased from 0.7 to 1.11 during the traditional germination ($p < 0.05$), while no significant changes were measured in germinated buckwheat by the ultrasound-assisted method. However, germination time

Table 1. Changes in color values of buckwheat during the germination

Germination	Time (h)	L*	a*	b*	C	ℎ	ΔE
Traditional	0	81.70±0.01[b]	1.9±0.02[a]	8.03±0.13[a]	8.25±0.14[a]	76.70±0.03[d]	-
	12	82.25±0.01[a]	1.74±0.05[a]	7.94±0.10[a]	8.12±0.10[a]	77.63±0.21[c]	0.44
	24	82.17±0.06[ab]	1.64±0.02[a]	7.88±0.11[a]	8.05±0.10[a]	78.20±0.31[b]	1.11
	48	82.66±0.19[a]	1.65±0.06[a]	8.23±0.25[a]	8.40±0.26[a]	78.61±0.07[b]	1.22
	72	82.16±0.33[ab]	1.54±0.11[a]	8.26±0.46[a]	8.40±0.48[a]	79.46±0.16[a]	2.29
Ultrasound-assisted	0	81.70±0.01[ab]	1.9±0.02[a]	8.03±0.13[a]	8.25±0.14[a]	76.70±0.03[d]	-
	12	81.15±0.50[b]	1.8±0.12[a]	7.91±0.40[a]	8.12±0.42[a]	77.15±0.23[cd]	0.40
	24	81.70±0.09[ab]	1.74±0.10[a]	7.96±0.39[a]	8.15±0.39[a]	77.68±0.08[c]	1.24
	48	81.35±0.01[b]	1.63±0.08[a]	8.13±0.39[a]	8.29±0.40[a]	78.63±0.01[b]	1.66
	72	81.99±0.06[a]	1.52±0.12[a]	8.18±0.38[a]	8.32±0.39[a]	79.49±0.36[a]	1.74

Data are shown as the mean±standard deviation. Different letters in the same row for buckwheat samples in each treatment that are

significantly different ($p < 0.05$)

and method significantly affected $b*$ values. The highest $b*$ values (11.00±0.22) were in the germinated sample by the traditional method. After 72 h, the $b*$ value was 9.87±0.14 in germinated buckwheat by ultrasound-assisted method. Color changes during germination were confirmed by the total color change (ΔE) values (*Table 1*). The results showed that ultrasound-assisted germination caused fewer color changes in Aktaş buckwheat than traditional germination. The total color changes above 3 are related to distinct color changes that can be detected by the human eye (Gu et al., 2023). However, the ΔE values were between 1 and 2 in germinated Aktaş buckwheat after 72 h. Thus, color changes were not detectable in the human eye during germination.
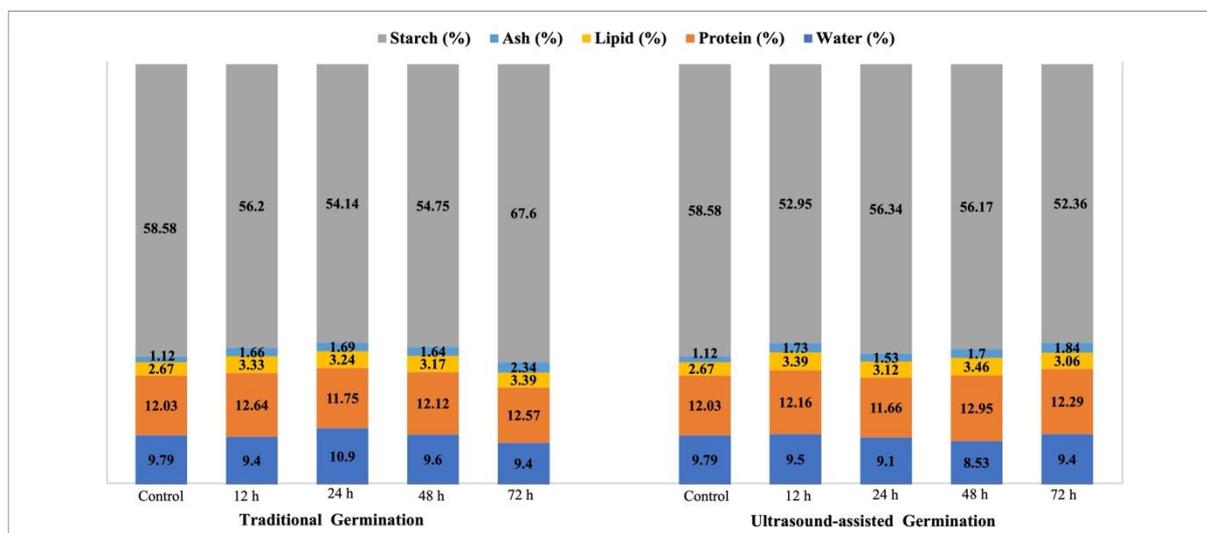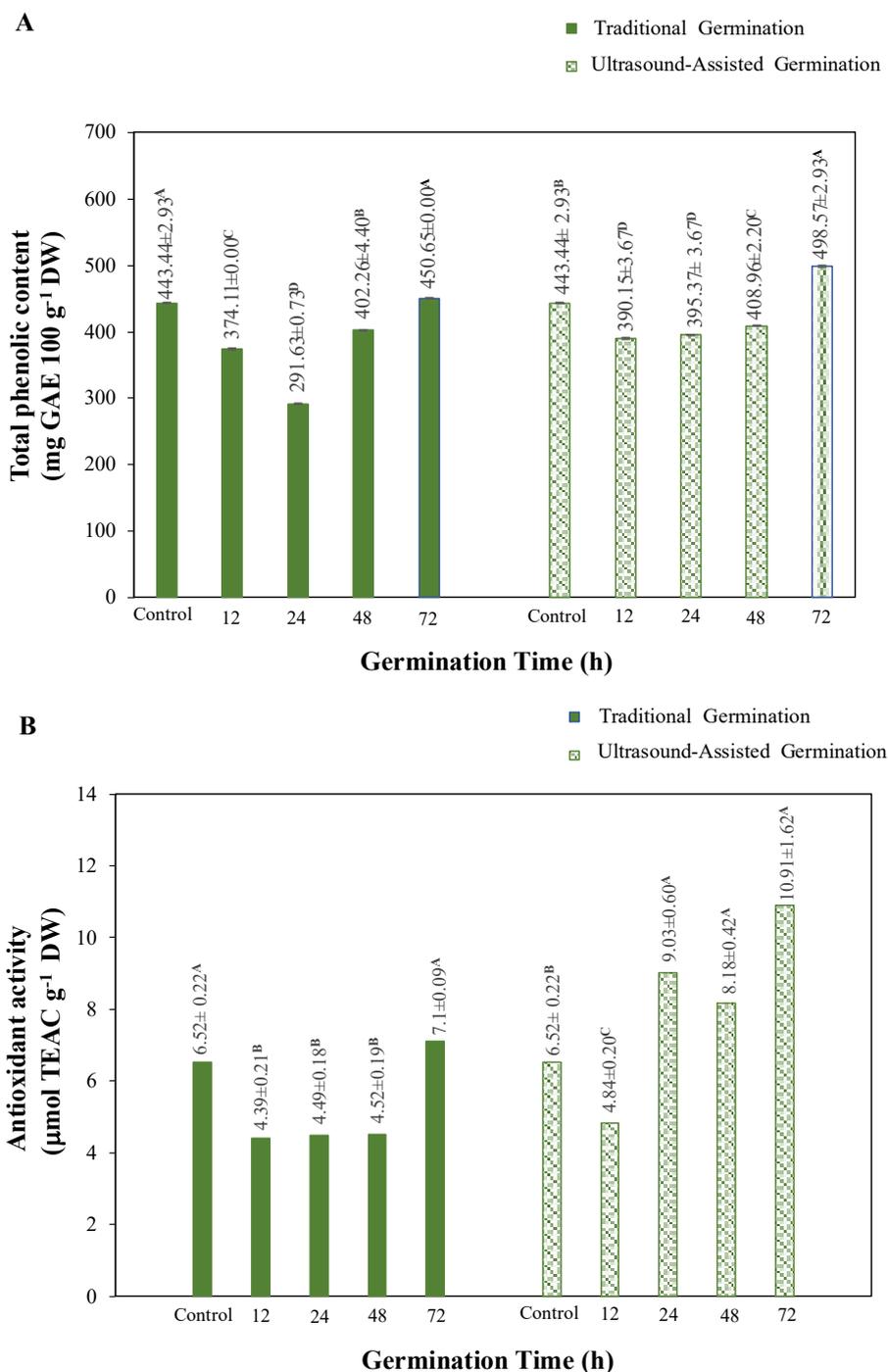

Figure 2. Changes in the proximate composition of buckwheat

3.3. Phenolic content and antioxidant activity

The changes in the antioxidant activity and phenolic content are shown in *Figure 3*. The total phenolic content (TPC) was 443.44±2.93 mg GAE 100 g^{-1} in raw buckwheat. Aktaş buckwheat had a higher phenolic content than tartary buckwheat (303 mg GAE 100 g^{-1}) (Zhang et al., 2015). During the first 12 h of germination, total phenolic content in Aktaş buckwheat decreased significantly ($p < 0.05$) compared to control sample. This reduction in TPC may result from the leaching of free phenolics into the steeping water. An increasing trend in TPC of buckwheat was observed with the increasing germination time for both germination methods (ultrasound assited and traditional). In the germinated samples using the ultrasound assisted method, the increase (12%) in TPC was significant ($p < 0.05$). However, the changes (7%) observed in the samples after germination of 72 h using the traditional method were not significant ($p > 0.05$). Similarly, previous studies have reported a significant improvement in the phenolic content of different varieties during germination (Bhinder et al., 2022; Thakur et al., 2021).

The antioxidant activity was 6.52±0.22 μmol TEAC g^{-1} in raw buckwheat and was significantly affected by germination time and method. The antioxidant activity of Aktaş buckwheat notably increased in germinated samples ($p < 0.05$). Ultrasound-assisted germinated samples showed a major increase (67%) in antioxidant activity after 72 hours. However, a slight increase was observed in the germinated sample by the traditional method after 72 h, similar to trend in TPC. In germinated grains, the accumulation and biosynthesis of phenolic compounds have been correlated to increased antioxidant activity (Bhinder et al., 2022). The increasing effects of germination on the antioxidant activity of buckwheat have been shown in previous studies (Aloo et al., 2021). Wang et al. (2020) evaluate effects of different treatments such as ultrasound, thermal stress and microwave on germination of tartary buckwheat. The ultrasonic and microwave treatments significantly increased the antioxidant activity when compared to the control sample. Similar to our study, the pretreatment method had a major effect on the antioxidant activity of germinated Tartary buckwheat.

A

B

Figure 3. Changes in the total phenolic content and antioxidant activity

(A: Changes in TPC and B: Changes in the antioxidant activity of buckwheat during germination)

3.4. Phytic acid and tannin content

Changes in the antinutrient content of buckwheat are shown in *Table 2*. The tannin content was 0.28 mg CE g^{-1}, and the phytic acid content was 11.1 ± 0.55 mg g^{-1} in raw buckwheat. Gemination significantly ($p < 0.05$) decreased ($43 – 54\%$) the tannin content, while the phytic acid content did not change dramatically ($p > 0.05$). A similar reduction (60%) in germinated buckwheat has been reported previously (Thakur et al., 2021). Kumari et al. (2023) reported a similar decrease (56%) in the tannin content after germination. However, tannins are valuable antioxidant sources that restrain the bioavailability of vitamin B_{12}, Fe^{+2}, and proteins in grains (Bhinder et al.,

2021). During germination, an increase in phytase enzyme activity causes a decrease in phytic acid concentration because of the changes in the structure of phytate phosphorus to inositol monophosphate (Kumari et al., 2023).

Table 2. Changes in antinutrient content of buckwheat during the germination

Germination	Time (h)	Tannin Content* (mg CE g^{-1})	Phytic Acid Content* (mg g^{-1})
Traditional	0	0.28±0.00[a]	11.1±0.55[ab]
	12	0.10±0.03[c]	10.29±0.80[b]
	24	0.15±0.07[bc]	10.75±0.33[ab]
	48	0.14±0.02[bc]	12.05±1.16[a]
	72	0.13±0.02[bc]	11.19±0.52[ab]
Ultrasound-assisted	0	0.28±0.00[a]	11.1±0.55[a]
	12	0.18±0.02[b]	9.48±0.42[ab]
	24	0.23±0.06[ab]	8.67±0.74[b]
	48	0.16±0.01[b]	10.77±0.15[a]
	72	0.16±0.02[b]	10.55±0.53[a]

* Data are presented as mean ±standard deviation. Different letters in the same row for buckwheat samples in each treatment indicate significant differences ($p < 0.05$).

4. Conclusions

Buckwheat is a valuable source of high-quality proteins, lipids, and starches. Germination significantly affected the nutritional and anti-nutritional properties of buckwheat ($p < 0.05$). Lipid and ash contents were higher in the germinated samples than in the control samples. The starch content decreased during germination because the starch molecules became more accessible to hydrolytic enzymes. During germination, the protein content can be changed by proteolysis or protein synthesis. Grain color can be impacted by germination. However, our findings demonstrated that germination had no adverse effect on Aktaş color values. Furthermore, the results indicated that germination is an effective way to mitigate antinutrients, such as tannins. Ultrasound-assisted germination effectively reached the maximum antioxidant activity and total phenolic content in Aktaş varieties. These results highlighted that germination methods have a major effect on the dynamic regulation of seed content in addition to germination time. Ultrasound-assisted germinated Aktaş buckwheat can be enhanced as a functional food ingredient with good nutritional and antioxidant qualities, in addition to a gluten-free structure.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Guzel, N.; Design: Guzel, N.; Data Collection or Processing: Altikardes, E.; Statistical analysis: Altikardes, E.; Literature Search: Altikardes, E., Guzel, N.; Writing, Review and Editing: Altikardes, E., Guzel, N.

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