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EDITED BY

Rakesh Kumar Gupta,
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REVIEWED BY

Paushali Mukherjee,
Indian Institute of Technology Guwahati,
India
Oludolapo Osunrinade,
Kwara State University, Nigeria

*CORRESPONDENCE

Amro B. Hassan
✉ ahassan2ks.c@ksu.edu.sa

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Induced germinated date (*Phoenix dactylifera*) seeds as a functional ingredient for wheat flour biofortification: effect of pretreatment on bioactive compounds and antioxidant activity

Latifah Saleh Altammami, Shahzad Hussain,
Mohammad A. Alshuniaber and Amro B. Hassan^{ID*}

Department of Food Sciences and Nutrition, College of Food and Agriculture Sciences, King Saud
University, Riyadh, Saudi Arabia

Introduction: The present study examined the phytochemical compounds and antioxidant properties of wheat flour supplemented with germinated date seed flour from the Khalas and Sagae cultivars.

Methods: Before germination, the seeds were soaked in different concentrations of citric acid (0%, 0.1%, 0.2%) for various durations (6, 12, and 24 hours) before germination. The partial least squares regression (PLS) model was applied to validate the pretreatment conditions. The optimised pretreatments, which promoted the accumulation of bioactive compounds and antioxidant capacity in germinated seeds, were 0.1% citric acid concentration and 6h soaking time for the Khalas cultivar, and 0.2% citric acid concentration and 6 h soaking time for the Sagae cultivar, respectively, applied for obtaining germinated date seed flour. The germinated seeds replaced wheat flour at levels of 2.5%, 5%, and 10%.

Results and discussion: Regarding the fortified wheat flour, findings stated that adding germinated date seed flour improved the bioactive compounds and antioxidant capacity. For example, the total phenolic content (TPC) increased from 9.0 mg GAE/g to 13.1 mg GAE/g (Khalas, 10%) and 31.2 mg GAE/g (Sagae, 10%). Similar trends were observed for total flavonoids, GABA, carotenoids, and antioxidant activities, as measured by ABTS, DPPH, and FRAP. Hence, substituting wheat flour with germinated date seed flour enhances the phytochemical content and antioxidant activity of the flour to acceptable levels, making it a promising ingredient to produce fortified flour in the food industry. Incorporating germinated date seed flour, obtained through optimised pretreatment, provides the food industry with an opportunity to develop functional bakery and cereal-based products with added health benefits. Furthermore, this approach promotes the sustainable utilisation of agricultural byproducts, aligning with current trends towards more sustainable and health-conscious food solutions.

KEYWORDS

antioxidants, biofortification, date seeds, germination, PLS validation

1 Introduction

Recently, date palm (*Phoenix dactylifera* L.) seeds have been recognised as a byproduct rather than agricultural waste (Kumari and Sana, 2024). It makes up, on average, 10%–15% of the mass of all date fruits, depending on the type, grade, and maturity. Date seed is typically used as a soil fertiliser or animal and poultry feed (Najjar et al., 2020), according to Al-Farsi and Lee (2008), date seeds contain a high quantity of 3.1%–7.1% moisture, 2.3%–6.4% protein, 5.0%–13.2% fat, 0.9%–1.8% ash, and 22.5%–80.2% dietary fibre and also a high level of antioxidants and phenolic compounds (3102–4,430 mg/100 g). However, it has been reported that the release of phenolics bound to the cell wall or the biosynthesis of phenolics may increase phytochemical levels during germination (Sritongtae et al., 2017). Additionally, the nutritional, functional and antioxidant properties of date seeds were significantly enhanced by traditional bioprocessing methods, such as germination (Kumari and Sana, 2024).

The germination process is an efficient and cost-effective bioprocessing technique in which enzymes are activated to decompose large-molecule nutrients into smaller constituents. Biochemical reactions associated with germination lead to the biosynthesis and accumulation of a diverse array of secondary metabolites, including flavonoids, vitamin C, tocotrienols, γ -aminobutyric acid, tocopherols, and phenolic compounds, while concurrently decreasing anti-nutritional factors such as phytic acid (Cankurtaran-Kömürcü and Bilgiçli, 2023; Kumari and Sana, 2024). Also, Randhir et al. (2004) stated that the germination process increases phenolic production, or that the release of cell wall-bound phenolics increases the phenolic content of seeds. However, based on previous investigations, pretreatment of grains and seeds significantly enhanced their antioxidant activity and phenolic compound levels. Sritongtae et al. (2017) stated that the pretreatment of soaking rice grains with 1% (w/v) citric acid increased their phenolic content, DPPH radical scavenging activity, and FRAP value. Moreover, pretreatment of pea seeds by soaking in acidified water containing different concentrations of salicylic acid for 24 h resulted in up to 11% increases in total phenolic content compared to unsoaked seeds. These findings might be due to a low-pH environment stimulating phenolic synthesis by acting as a signal to initiate defence response genes (McCue et al., 2000).

In wheat milling, to produce refined wheat flour, the bran and part of the germ are removed. Removing these parts reduces the nutritional value of wheat flour because they are rich in antioxidants and phenolic compounds, such as ferulic and caffeic acids, as well as other substances, including phytic acid, vitamin E, and selenium. Hence, the milling industry has been forced to enrich wheat flour with natural ingredients containing vitamins and minerals. Accordingly, more research is required to enhance the nutritional value of wheat flour derived from natural sources (Brewer et al., 2024). Adding date seed flour or phenolic extracts as byproduct ingredients can enrich foods with a range of nutritional and functional properties. It has been reported that adding date seeds to wheat flour enhances its nutritional quality (Ahmed et al., 2024). The experiments generally showed that adding flour from germinated legumes improved the rheological properties of the dough samples. For instance, the addition typically results in higher water absorption values. However, some varieties of flour made from germinated legumes have also been found to exhibit lower water absorption when added to bread recipes (Atudorei and Codină, 2020).

Despite date seed flour or its phenolic extracts being reported to enhance the nutritional quality of wheat flour (Ahmed et al., 2024).

However, fortifying wheat flour with germinated date seeds has not yet been investigated. According to improvement, it results from germination. The addition of germinated date seeds might significantly improve the physicochemical, antioxidant, and techno-functional properties of wheat flour without adversely affecting the final product's sensory attributes. Hence, the purpose of this study is to investigate the synergistic effects of citric acid concentration and soaking time as pretreatments on germination efficiency and the bioactive compounds and antioxidant activity in two date cultivars (Khalas and Sagae), as well as to evaluate the effects of combining germinated date seed flour with wheat flour on its physicochemical and nutritional characteristics of the resultant composite flour. Thus, this study examines the potential of germinated date seed flour to provide insights into utilising this underutilised ingredient to improve the nutritional content and overall rheological quality of wheat-based products.

2 Materials and methods

2.1 Date seed collection and preparation

Date seeds collected from local Saudi date cultivars Khalas and Sagae were obtained from the date production plants of the Ministry of Environment, Water, and Agriculture, Saudi Arabia. The seeds were collected from the mature date fruits for each cultivar. Also, only whole and undamaged seeds were included to ensure the quality and consistency of the samples. The collected seeds were thoroughly cleaned by manually removing adhering residues, followed by washing with distilled water to remove surface impurities. The cleaned seeds were subsequently dried at ambient temperature.

2.2 Date seed germination and flour preparation

Before germination, approximately 300 grams of seeds from each cultivar were soaked (1:3, w/v) for 6 h in citric acid water at 0.1% for Khalas seeds and 0.2% for Sagae seeds (Sritongtae et al., 2017). The soaking period and citric acid concentrations were applied according to our primary validation study of germination rate and phytochemical compounds content. After soaking, the seeds were rinsed under tap water and washed with distilled water. The seeds were kept on a wet muslin cloth covered with plastic wrap and placed in a dark place, with frequent monitoring and watering, at 25 °C (± 2) and 70 $\pm$ 5% relative humidity for 3 weeks, as described by Kumari and Sana (2024). The germinated seeds were dried at 45 °C for 24 h, then ground into a fine powder using a laboratory grinder and sieved to a particle size of less than 0.5 mm. The germinated date seed flour was packaged in sealed polyethylene bags and refrigerated at 4 °C until further analysis. The replacement rates of date seed flour to wheat flour (2.5, 5, and 10%) were selected based on preliminary trials made from low (less than 10%) and higher (up to 15%) replacement levels and also, as supported by the literature conducted by Mrabet et al. (2024) and El-Salam et al. (2024).

2.3 Determination of colour

The colour of the germinated date seed flour was estimated to evaluate L* (whiteness), a* (red/green), and b* (yellow/blue) values

using a colourimetric apparatus (Hunter Lab Colour Flex 150 Hunter Associates Inc., USA). Colour variations were calculated using the following equation:

$$\Delta E = \sqrt{\left\{ \left(L_2^* - L_1^* \right)^2 + \left(a_2^* - a_1^* \right)^2 + \left(b_2^* - b_1^* \right)^2 \right\}}$$

2.4 Bioactive compounds and antioxidant activity

2.4.1 Gamma-aminobutyric acid content

The γ -Aminobutyric Acid (GABA) content was determined as described by [Sansenya et al. \(2017\)](#). The control date seed flour and germinated date seed flour (2 g) were extracted with deionised water (15 mL), then centrifuged at 10,000 rpm for 15 min. The supernatant was filtered (0.45 μ m), and 0.5 mL of the filtrate was reacted with borate buffer (pH 9.0), phenol reagent, and sodium hypochlorite. After boiling for 10 min and cooling, the absorbance was measured at 645 nm using a UV–Vis spectrophotometer. A standard curve was prepared using known concentrations of GABA, and sample concentrations were calculated from the regression equation of the standard curve ($R^2 = 0.9825$).

2.4.2 Determination of carotenoids

Total carotenoids were determined based on the procedure described by [Jacques et al. \(2009\)](#). Briefly, acetone (25 mL) was used to extract carotenoids from control date seed flour and germinated date seed flour (1 g). The extract was then partitioned with petroleum ether and distilled water to separate the carotenoid-rich organic phase. The absorbance of the upper layer was measured at 450 nm using a UV–Vis spectrophotometer (PD-303 UV). The carotenoid content was calculated and expressed as micrograms per gram of dry matter (μ g/g DM).

2.4.3 Determination of anthocyanin content

The anthocyanin content was determined according to the methodology outlined by [Lapornik et al. \(2005\)](#). Anthocyanins were extracted from control and germinated date seed flour samples using a 70% ethanolic solvent. The extract was subsequently treated with an ethanol–HCl solution (0.01% HCl), a 2% HCl solution (pH 0.8), and a citric buffer (pH 3.5). The 70% ethanol solution was used as a neutral reference at 520 nm. The anthocyanin content was expressed as mg /g.

2.5 Extraction for determination of phenolic, flavonoid and antioxidant activities

Extracts of phenolics and antioxidants from the control and germinated date seed flour were prepared according to the method described by [Talhaoui et al. \(2014\)](#). Briefly, seed flour (10 g) was mixed with absolute methanol (100 mL) at a 1:10 (w/v) ratio and shaken at room temperature (20 °C) for 24 h. The mixture was then centrifuged at 10,000 rpm for 15 min, and the supernatant was filtered, collected and stored at 4 °C until analysis.

2.5.1 Determination of total phenolic content

The total phenolic content (TPC) was measured using the Folin–Ciocalteu protocol as described by [Waterhouse \(2002\)](#). Briefly, 100 μ L of each extract was mixed with 1.5 mL of distilled water and 100 μ L of Folin–Ciocalteu reagent. After 8 min of incubation at room temperature, 300 μ L of 7.5% (w/v) sodium carbonate solution was added. The mixture was incubated in the dark at room temperature for 2 h, then diluted to 5 mL with distilled water. The absorbance was measured at 765 nm using a UV–Vis spectrophotometer. Gallic acid was used to construct the standard calibration curve, and results were expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g).

2.5.2 Determination of flavonoid content

The colourimetric method reported by [Kim et al. \(2003\)](#) was used to quantify the total flavonoid content (TFC) of the flour samples. In Falcon tubes (10 mL), 1 mL of extract was combined with 4 mL of distilled water and 0.3 mL of 5% NaNO_2 solution. After letting the tubes stand for 5 min, 0.3 mL of 10% AlCl_3 was added to the mixture, which was then left to stand for an additional 6 min. Finally, 2.4 mL of distilled water and 2 mL of 1 N NaOH were added, and everything was well mixed. The tubes were then left at room temperature for 15 min in a dark location, and the absorbance was measured relative to a blank prepared similarly by substituting methanol for the mixture using a UV spectrophotometer at 510 nm. A standard curve of catechin (mg CE/g) was used to quantify the total flavonoid content.

2.5.3 DPPH scavenging activity

Antioxidant activity was assessed using the DPPH radical scavenging method described by [Chang et al. \(2001\)](#), as cited in [Shyura et al. \(2005\)](#), with slight modifications. The reaction mixture (3 mL) consisted of 1.0 mL of 0.1 mM DPPH–ethanol solution, 0.9 mL of 50 mM Tris–HCl buffer (pH 7.4), and 0.1 mL of sample solution (5.0 mg/mL). A blank and control were prepared using methanol in place of the DPPH solution or sample. After 30 min of incubation in the dark at room temperature, absorbance was measured at 517 nm. Antioxidant capacity was quantified using a Trolox standard curve and expressed as mg Trolox equivalents per gram of sample (mg TE/g).

2.5.4 Ferric-reducing antioxidant power

Composite Flours were tested for antioxidant capacity using the ferric reducing antioxidant power (FRAP) assay. The Fe^{3+} /triipyridyl triazine complex, which is present in stoichiometric excess, is reduced by reductants (antioxidants) in the sample in the FRAP assay to the blue-colored ferrous form, with an increase in absorbance at 593 nm, according to the procedure outlined by [Benzie and Strain \(1996\)](#). A standard curve using Trolox was constructed, and FRAP values were expressed as mg Trolox equivalents per gram of sample (mg TE/g).

2.5.5 ABTS scavenging determination

The 100 mL ABTS stock solution was prepared with distilled water containing ABTS (7 mM) and 2.45 mM potassium persulfate. After that, it was left at room temperature in the dark for 12 to 16 h. The solution was diluted with phosphate buffer solution (pH 7) to

have an absorbance of 0.7 ± 0.5 at 734 nm before use. Then, the ABTS scavenging ability was measured by mixing 50 μL of the extracts with 1.950 mL of the ABTS solution, and the absorbance was measured at 734 nm (Re et al., 1999). A standard curve of Trolox (mg TE/g) was used to quantify the ABTS scavenging activity.

2.6 Statistical analysis

All experimental treatments were arranged in a factorial design, considering two main factors: date seed cultivar and the level of germinated date seed flour supplementation in wheat flour. Each treatment combination was independently replicated in three separate batches (biological replicates), and all measurements were conducted in triplicate (technical replicates). Results are presented as mean $\pm$ standard deviation (SD). Data was statistically analysed using the XLSTAT Premium software package (version 2023.2). Two-way analysis of variance (ANOVA) was performed to assess the main effects of the factors as well as their interactions. When significant differences were detected, mean comparisons were conducted using the least significant difference (LSD) test at a 5% significance level. Additionally, multivariate analyses, including HJ-biplot principal component analysis (PCA) and partial least squares regression (PLS), were performed using XLSTAT, following the approaches described by Vidal et al. (2020) and Tenenhaus et al. (2005), respectively. The PLS validation model explains X of the variance in the predictors and Y of the variance in the response variables. The predictive ability was assessed using cross-validation, confirming the model's robustness.

3 Results and discussion

3.1 Colour of germinated seeds

Table 1 illustrates the impact of pretreatment of soaking date seeds in citric acid solution for different periods on the colour parameters (L^* , a^* , b^* , and ΔE) of germinated date seeds from Khalas and Sagae cultivars. The colour parameters indicate variations in lightness (L^*), redness/greenness (a^*), yellowness/blueness (b^*), and the overall colour difference (ΔE) in germinated date seeds resulting from various pretreatments. For lightness (L^*), both cultivars showed a tendency towards lower values than the control, with Khalas ranging from 32.28 to 42.86 and Sagae from 35.81 to 41.17. The highest L^* value was observed in the control samples, whereas lower values were generally associated with higher citric acid concentrations and longer soaking durations.

Two-way ANOVA revealed a highly significant impact of citric acid concentration on L^* in the Sagae cultivar. However, the a^* and b^* values in germinated date seeds, indicating redness and yellowness respectively, varied among interventions but did not exhibit any significant overall trends. The maximum a^* values were recorded at 0.1% citric acid after 12 h of pretreatment for both cultivars, while the b^* values remained relatively stable, exhibiting only slight decreases compared to the control. The overall colour difference (ΔE) increased following treatment in both cultivars, indicating a perceptible colour change relative to the control. The maximum ΔE values were observed at 0.2% citric acid after 12 h for Khalas (10.37) and at 0.1% citric acid after 24 h for Sagae (5.85), indicating that citric acid concentration and

TABLE 1 Colour of germinated date seeds cultivars affected by citric acid concentrations and soaking times.

Citric acid concentration	Soaking time	Cultivars							
		L^*		a^*		b^*		ΔE	
		Khalas	Sagae	Khalas	Sagae	Khalas	Sagae	Khalas	Sagae
Control		$42.8 \pm 2.49^{a(*)}$	40.7 ± 1.08^{AB}	8.8 ± 1.90^a	10.6 ± 0.68^A	29.6 ± 1.55^a	25.4 ± 0.92^D	0.0 ± 0.0^c	0.0 ± 0.00^D
0%	6 h	38.7 ± 0.73^{bcd}	38.6 ± 1.16^{ABCD}	7.4 ± 0.74^a	10.8 ± 1.08^A	26.4 ± 0.37^{bc}	26.5 ± 0.70^{BCD}	5.1 ± 1.80^b	4.0 ± 2.12^{ABC}
	12 h	37.6 ± 2.89^{bcd}	38.2 ± 0.53^{CDE}	7.8 ± 2.17^a	11.3 ± 0.84^A	27.5 ± 0.42^{abc}	26.6 ± 0.29^{BCD}	5.4 ± 2.54^b	3.2 ± 0.31^{BC}
	24 h	37.4 ± 1.40^{bcd}	37.1 ± 0.97^{DE}	8.5 ± 2.63^a	10.9 ± 1.76^A	27.1 ± 0.95^{bc}	25.5 ± 0.32^{CD}	6.0 ± 0.65^b	5.7 ± 0.43^{AB}
0.1%	6 h	40.2 ± 2.50^{abc}	35.8 ± 3.06^E	7.8 ± 1.17^a	11.8 ± 1.86^A	28.6 ± 0.67^{ab}	26.1 ± 1.63^{BCD}	5.1 ± 2.43^b	5.6 ± 3.19^{AB}
	12 h	38.1 ± 2.80^{bcd}	37.8 ± 0.96^{CDE}	11.2 ± 2.88^a	12.8 ± 2.24^A	26.5 ± 2.34^{bc}	27.2 ± 0.32^{AB}	5.1 ± 3.04^b	5.8 ± 0.63^{AB}
	24 h	36.9 ± 1.89^{cd}	36.2 ± 0.21^{DE}	9.5 ± 1.03^a	11.2 ± 1.34^A	26.9 ± 1.91^{bc}	25.4 ± 0.17^D	6.4 ± 3.46^b	5.8 ± 2.25^A
0.2%	6 h	35.5 ± 2.45^{de}	38.5 ± 0.82^{BCD}	10.4 ± 4.51^a	11.0 ± 0.79^A	25.5 ± 1.89^c	26.7 ± 0.57^{BC}	8.4 ± 1.08^{ab}	2.8 ± 0.60^C
	12 h	32.3 ± 1.40^e	41.1 ± 0.21^A	9.1 ± 3.22^a	11.4 ± 0.72^A	26.2 ± 1.42^c	28.4 ± 0.40^A	10.3 ± 1.65^a	2.8 ± 0.41^C
	24 h	41.1 ± 2.94^{ab}	39.7 ± 1.01^{ABC}	9.5 ± 0.24^a	10.9 ± 0.39^A	28.5 ± 0.54^{ab}	27.1 ± 0.53^B	6.1 ± 0.05^b	2.4 ± 1.44^{CD}
Two-way ANOVA									
Salt concentration (C)		*	***	Ns	Ns	*	**	*	**
Soaking time (ST)		*	*	Ns	Ns	*	**	Ns	Ns
C* ST		**	*	Ns	Ns	Ns	Ns	Ns	Ns
SE $\pm$		1.308	0.861	1.382	0.753	0.798	0.419	1.256	0.876
LSD		3.859	2.54	4.076	2.221	2.353	1.236	3.441	2.583

Data were evaluated using a two-way ANOVA, with soaking time and citric acid concentration as factors for each date-seed cultivar. Capital caps letters indicate significant differences among the Sagae cultivar, whereas lower caps letters indicate significant differences among the Khalas cultivar. Within the column means with identical letters indicate that the values do not differ significantly at $p < 0.05$, according to LSD. Asterisks (*) indicate significantly influential factors as follows: ns, not significant; **, significant at $p \leq 0.01$; ***, significant at $p \leq 0.001$ level. Each value represents the average of the three replicates.

immersion duration can significantly influence colour changes in germinated seeds. Cankurtaran-Kömürçü and Bilgiçli (2023) similarly found that applying organic acids to germinated wheat and ancient grains significantly affected their colour properties. As documented by Baranzelli et al. (2018), germination led to a significant decrease in the flour's luminance (L^*) value, resulting in a darker appearance. There was also an increase in value, indicating a shift toward a redder colouration, while the b^* values showed minimal variation, except in the IG48 treatment. These findings suggest that germination causes the flour to become darker and slightly redder. The change in colour of the sample, mainly due to non-enzymatic browning and/or the Maillard reaction, may result in the decomposition of colour pigments, and non-enzymatic reactions may produce darkness in germinated date seed flour (Soysal et al., 2009).

3.2 Phytochemical compounds of germinated seeds

Table 2 summarises the influence of soaking date seeds (0, 6 and 12 h) in citric acid solution (0, 0.1, and 0.2%) on the levels of GABA, anthocyanins, and carotenoids (measured as β -carotene) in germinated date seeds of the Khalas and Sagae cultivars. As shown in the table, the GABA, anthocyanins, and carotenoids content of the raw date seeds were found to be 17.6 mg/g, 4.45 mg/g, 2.48 mg β -carotene/g for Khalas and 13.88 mg/g, 4.17 mg/g, 1.13 mg β -carotene/g Sagae cultivar, respectively. The results demonstrate considerable variation in GABA, anthocyanin, and carotenoid levels

across cultivars, citric acid concentrations, and soaking durations. GABA is a bioactive compound that plays an essential role in neurotransmission and has been associated with neuroprotective effects and reduced stress and anxiety (Gan et al., 2017). Regarding GABA content, seed germination caused a significant change ($p < 0.05$) in both cultivars. However, in the Sagae date seeds, it was significantly increased, and the highest GABA level was obtained after soaking for 12 h in citric acid solution at the concentrations 0% (17.79 mg/g), 0.1% (17.52 mg/g), and 0.2% (17.54 mg/g), respectively. Whereas, soaking Khalas seeds in 0.2% citric acid solution for 6 and 12 h showed significant ($p < 0.05$) increases in GABA content to 17.70 and 17.91 mg/g, respectively. The two-way ANOVA revealed that citric acid concentration and its interaction with soaking time significantly influenced GABA content ($p < 0.01$ or $p < 0.001$), particularly in Sagae. These findings are consistent with previous research, which indicates that germination is among the most effective methods for synthesising GABA in cereals, as it activates glutamic acid decarboxylase (GAD). During germination, the GABA levels in grains such as brown rice, wheat, and soybeans were found to increase significantly (Yen et al., 2022). Anthocyanins are potent antioxidants with anti-inflammatory properties and have been linked to improved cardiovascular health and reduced chronic disease risk (Yunusa et al., 2025). Anthocyanin content showed greater variation, particularly in the Khalas cultivar, where levels ranged from 4.45 mg/g in control to 18.64 mg/g in germinated seeds soaked for 12 h in a 0.1% citric acid solution. Similarly, the anthocyanin content of germinated Sagae cultivar seeds was high after 6 h of pretreatment with 0.2% citric acid

TABLE 2 Bioactive compounds (GABA, Anthocyanin, carotenoid) of germinated date seeds cultivars affected by citric acid concentrations and soaking time.

Salt concentration		Soaking time	GABA (mg/g)		Anthocyanin (mg/g)		Carotenoid (mg β -carotene/g)	
			Cultivars					
			Khalas	Sagae	Khalas	Sagae	Khalas	Sagae
Control			17.60 $\pm$ 0.149 ^{bc}	13.88 $\pm$ 0.45 ^E	4.45 $\pm$ 0.96 ^c	4.17 $\pm$ 0.00 ^{BCD}	2.48 $\pm$ 0.29 ^a	1.13 $\pm$ 0.074 ^E
0%	6 h		17.59 $\pm$ 0.064 ^{bc}	14.38 $\pm$ 0.10 ^D	12.24 $\pm$ 7.65 ^{ab}	3.06 $\pm$ 2.41 ^{CD}	1.17 $\pm$ 0.31 ^{bc}	4.73 $\pm$ 0.00 ^A
	12 h		16.64 $\pm$ 1.719 ^{bc}	17.79 $\pm$ 0.02 ^A	9.74 $\pm$ 0.48 ^{bc}	9.18 $\pm$ 1.45 ^{AB}	1.19 $\pm$ 0.44 ^{bc}	2.51 $\pm$ 0.174 ^C
	24 h		17.47 $\pm$ 0.021 ^c	15.58 $\pm$ 0.15 ^C	15.30 $\pm$ 1.93 ^{ab}	5.84 $\pm$ 1.45 ^{BCD}	0.63 $\pm$ 0.13 ^{cd}	1.16 $\pm$ 0.251 ^E
0.1%	6 h		17.57 $\pm$ 0.149 ^{bc}	11.73 $\pm$ 0.59 ^G	11.68 $\pm$ 1.67 ^{abc}	8.07 $\pm$ 8.19 ^{BC}	0.56 $\pm$ 0.31 ^{cd}	4.77 $\pm$ 0.442 ^A
	12 h		17.46 $\pm$ 0.170 ^c	17.52 $\pm$ 0.06 ^A	18.64 $\pm$ 10.06 ^a	14.74 $\pm$ 2.41 ^A	1.40 $\pm$ 0.42 ^b	0.53 $\pm$ 0.00 ^F
	24 h		17.52 $\pm$ 0.191 ^{bc}	15.77 $\pm$ 0.08 ^C	16.69 $\pm$ 2.50 ^{ab}	5.84 $\pm$ 1.45 ^{BCD}	1.27 $\pm$ 0.95 ^{bc}	0.90 $\pm$ 0.390 ^{EF}
0.2%	6 h		17.70 $\pm$ 0.191 ^{ab}	12.59 $\pm$ 0.06 ^F	10.85 $\pm$ 1.45 ^{bc}	14.19 $\pm$ 2.89 ^A	0.40 $\pm$ 0.22 ^d	3.81 $\pm$ 0.301 ^b
	12 h		17.91 $\pm$ 0.058 ^a	17.54 $\pm$ 0.04 ^A	13.35 $\pm$ 1.67 ^{ab}	6.12 $\pm$ 3.85 ^{BCD}	0.69 $\pm$ 0.42 ^{bcd}	1.78 $\pm$ 0.00 ^D
	24 h		17.62 $\pm$ 0.106 ^{bc}	16.66 $\pm$ 0.02 ^B	10.02 $\pm$ 1.67 ^{bc}	1.67 $\pm$ 0.00 ^D	0.92 $\pm$ 0.29 ^{bcd}	0.69 $\pm$ 0.028 ^F
Two-way ANOVA								
Salt concentration (C)			**	***	**	*	**	***
Soaking time (ST)			Ns	***	**	**	**	***
C* ST			Ns	***	**	**	*	***
SE $\pm$			0.075	0.142	2.470	1.899	0.249	0.134
LSD			0.223	0.418	7.285	5.602	0.735	0.395

Data were evaluated using a two-way ANOVA, with soaking time and citric acid concentration as factors for each date-seed cultivar. Capital caps letters indicate significant differences among the Sagae cultivar, whereas lower caps letters indicate significant differences among the Khalas cultivar. Within the column means with identical letters indicate that the values do not differ significantly at $p < 0.05$, according to LSD. Asterisks (*) indicate significantly influential factors as follows: ns, not significant; **, significant at $p \leq 0.01$; ***, significant at $p \leq 0.001$ level. Each value represents the average of the three replicates.

(14.19 mg/g). ANOVA analysis demonstrated significant effects of the pretreatment process (soaking time) and citric acid concentration, as well as their interaction, on the anthocyanin content of germinated Sagae seeds. Fan et al. (2024) reported that germination leads to a significant increase in anthocyanin content in red rice. This finding is consistent with the results of the present study, which showed notable increases in anthocyanin levels in both Khalas and Sagae cultivars under specific soaking times and citric acid concentrations. Carotenoids, such as β -carotene, are well known for their antioxidant activity, support for eye health, and contribution to immune system function (Maqsood et al., 2020). As shown in Table 2, the carotenoid content was 2.48 and 1.13 mg β -carotene/g in Khalas and Sagae raw date seeds, respectively. It was observed that germination of Khalas date seeds significantly reduced carotenoid levels, with the lowest values observed at the highest citric acid concentration (0.2%). In contrast, a substantial increase in carotenoid content was observed in germinated Sagae seeds, especially in those soaked for 6 h in citric acid solutions at concentrations of 0.1 and 0.2%, reaching 4.77 and 3.81 mg β -carotene/g, respectively. These findings align with prior research, which documented considerable increases in carotenoid and anthocyanin content in date seed flour during germination (Yunusa et al., 2025). However, some variability was observed across germination stages. The increase in anthocyanin content observed in this study may be attributed to an antioxidant response during the initial metabolic phases. At the same time, fluctuations may result from changes in metabolic priorities as seeds transition from dormancy to active growth (Yunusa et al., 2025). Furthermore, the observed effects of citric acid concentration and immersion duration underscore the

significance of optimising germination conditions to enhance the nutritional and functional properties of date seeds.

Table 3 shows the total phenolic content (TPC) and total flavonoid content (TFC) of germinated date seeds from the Khalas and Sagae cultivars pre-treated with soaking in citric acid solution (0, 0.1, and 0.2%) for different times (6, 12, and 24 h). Both phenolic and flavonoid compounds exhibit strong antioxidant properties and can help protect cells from oxidative stress, contributing to overall health (Zarie et al., 2023). The data reveal significant differences in TPC and TFC across citric acid concentration, soaking time, and cultivar. For TPC, both cultivars showed notable increases following citric acid applications at 0.1% and 0.2%, especially after 6 h of soaking. The TPC values in Khalas rose from 46.03 mg GAE/g in the control to 61.54 mg GAE/g at 0.2% citric acid after 6 h, while Sagae exhibited the highest TPC (78.31 mg GAE/g) at 0.2% citric acid following 12 h of soaking.

Regarding TFC, the values remained comparatively stable across the interventions, with minor, predominantly non-significant variations. For Khalas, the TFC ranged from 7.54 to 10.41 mg CE/g, whereas for Sagae, it ranged from 7.32 to 9.50 mg CE/g. As shown in the two-way ANOVA table, citric acid concentration, soaking time, and their interaction had highly significant effects ($p < 0.001$) on most of the measured parameters, except TFC in the Sagae cultivar.

These findings are consistent with those reported by Rajhi et al. (2024), who observed a substantial increase in TPC and TFC in germinated faba bean seeds. They stated that the TPC and TFC increased by 3.61- and 22-fold, respectively. These results substantiate that the germination process, particularly when combined with pretreatment soaking in a citric acid solution, significantly improves phenolic and

TABLE 3 Total phenolic content (TPC) and total flavonoid content (TFC) of germinated date seeds cultivars affected by citric acid concentrations and soaking times.

Citric acid concentration		Soaking time	TPC (mg GAE/g)		TFC (mg CE/g)	
			Cultivars			
			Khalas	Sagae	Khalas	Sagae
Control			46.03 ± 1.37 ^c	58.40 ± 0.14 ^D	10.41 ± 1.32 ^a	8.68 ± 0.38 ^{AB}
0%	6 h		57.78 ± 3.52 ^b	76.50 ± 1.28 ^{AB}	9.45 ± 0.59 ^b	8.27 ± 0.73 ^{BC}
	12 h		45.46 ± 1.21 ^c	73.65 ± 1.88 ^B	9.57 ± 0.21 ^{ab}	7.32 ± 0.00 ^C
	24 h		52.00 ± 0.03 ^d	75.95 ± 0.84 ^{AB}	7.81 ± 0.16 ^c	9.02 ± 0.67 ^{AB}
0.1%	6 h		61.1 ± 2.18 ^a	75.85 ± 1.70 ^{AB}	9.67 ± 0.61 ^{ab}	8.90 ± 0.68 ^{AB}
	12 h		54.40 ± 0.12 ^{cd}	67.18 ± 5.14 ^C	7.54 ± 0.03 ^c	8.45 ± 0.68 ^{ABC}
	24 h		40.30 ± 0.89 ^f	65.67 ± 0.43 ^C	7.84 ± 0.00 ^c	8.73 ± 0.95 ^{AB}
0.2%	6 h		61.54 ± 0.00 ^a	76.18 ± 0.12 ^{AB}	9.22 ± 0.35 ^b	8.06 ± 0.63 ^{BC}
	12 h		32.61 ± 0.00 ^g	78.31 ± 3.98 ^A	9.92 ± 0.15 ^{ab}	9.50 ± 0.79 ^A
	24 h		40.81 ± 4.89 ^f	54.55 ± 2.22 ^D	7.57 ± 0.12 ^c	8.48 ± 0.81 ^{AB}
Two-way ANOVA						
Citric acid concentration (C)			***	***	*	*
Soaking time (ST)			***	***	***	ns
C* ST			***	***	***	*
SE±			1.228	1.369	0.300	0.392
LSD			3.623	4.038	0.886	1.157

Data were evaluated using a two-way ANOVA, with soaking time and citric acid concentration as factors for each date-seed cultivar. Capital caps letters indicate significant differences among the Sagae cultivar, whereas lower caps letters indicate significant differences among the Khalas cultivar. Within the column means with identical letters indicate that the values do not differ significantly at $p < 0.05$, according to LSD. Asterisks (*) indicate significantly influential factors as follows: ns, not significant; **, significant at $p \leq 0.01$; ***, significant at $p \leq 0.001$ level. Each value represents the average of the three replicates.

flavonoid content across various seed varieties. Furthermore, the present findings indicate that prolonged soaking may lead to the degradation or leaching of phenolic compounds, as reported in studies on some legumes and cereals (Sritongtae et al., 2017; Randhir et al., 2004).

3.3 Antioxidant activity of germinated date seeds

Table 4 illustrates the influence of citric acid concentration and soaking time on the antioxidant capacities of germinated date seeds from Khalas and Sagae cultivars, as determined by ABTS, DPPH, and FRAP assays. The data demonstrate that antioxidant activity fluctuates with cultivar, citric acid concentration, and soaking time in the germinated seeds. For the ABTS assay, values remained comparatively stable across treatments in both cultivars, with Khalas ranging from 5.09 to 5.16 mg TE/g and Sagae from 4.95 to 5.17 mg TE/g. The highest values were recorded at 0.1 and 0.2% citric acid after 12 and 24 h of soaking. Soaking duration exerted a significant influence ($p < 0.001$) on ABTS activity of the germinated seeds, whereas citric acid concentration did not have a substantial effect in each cultivar.

The DPPH assay showed greater significance ($p < 0.001$), particularly in Khalas, where values increased from 1.55 mg TE/g in raw seeds to a peak of 4.82 mg TE/g in germinated seeds soaked in 0.1% citric acid for 12 h. Similarly, germinated Sagae seeds showed a significant increase in DPPH scavenging activity, reaching a maximum value of 3.45 mg TE/g after 6 h of soaking in a 0.2% citric acid solution. Statistically significant effects ($p < 0.01$ or $p < 0.001$) were observed for citric acid concentration and soaking time in the Sagae cultivar, with

notable interactions in both cultivars. As for FRAP activity, both cultivars showed enhanced antioxidant capacity after germination, especially at 12 and 24 h of immersion, with Khalas reaching 49.86 mg TE/g and Sagae up to 53.30 mg TE/g. Citric acid concentration and immersion duration significantly impacted FRAP values ($p < 0.01$ or $p < 0.001$), with a notable interaction effect in Khalas. These findings agree with previous reports documenting increases in antioxidant capacity in germinated legumes and cereals (Atudorei and Codină, 2020).

According to Siriparu et al. (2022), germination significantly enhances antioxidant activity in mung bean seeds, as evidenced by a 2–3-fold increase in ABTS activity and a 1.46–1.82-fold increase in DPPH activity from the third day of germination. Moreover, Khang et al. (2016) demonstrated that germination enhances the antioxidant activity of legumes. Their findings revealed that extracts from germinated legumes showed varying yet elevated antioxidant activity, as assessed by DPPH and reducing power assays. The result confirms that the germination process enhances antioxidant capacity.

3.4 Validation models

The validation model demonstrated the synergistic interaction between the pretreatment methods and the phytochemical compounds, as well as the antioxidant activity and colour properties of germinated date seeds from the Khalas and Sagae cultivars. These validation models were evaluated using Principal Component Analysis (PCA) and Partial Least Squares (PLS). The PCA for Sagae date seeds and Khalas date seeds were conducted in Figures 1B, 2A,

TABLE 4 Antioxidant activity (ABTS, DPPH, FRAP) of germinated date seeds cultivars affected by citric acid concentrations and soaking time.

Salt concentration		Soaking time	ABTS (mg TE/g)		DPPH (mg TE/g)		FRAP (mg TE/g)	
			Cultivars					
			Khalas	Sagae	Khalas	Sagae	Khalas	Sagae
Control			5.16 ± 0.00 ^a	4.95 ± 0.14 ^B	1.55 ± 0.73 ^s	2.41 ± 0.14 ^{CD}	46.47 ± 1.64 ^d	46.67 ± 1.51 ^D
0%	6 h		5.12 ± 0.02 ^c	4.95 ± 0.03 ^B	2.91 ± 0.14 ^{def}	3.31 ± 0.45 ^A	18.14 ± 0.36 ^e	48.57 ± 2.11 ^{CD}
	12 h		5.15 ± 0.01 ^b	5.13 ± 0.00 ^A	3.33 ± 0.24 ^{cd}	3.33 ± 0.28 ^A	49.08 ± 0.84 ^a	51.22 ± 2.14 ^{AB}
	24 h		5.12 ± 0.01 ^c	5.09 ± 0.01 ^A	4.05 ± 0.28 ^b	2.65 ± 0.14 ^{BC}	49.86 ± 0.28 ^a	53.30 ± 0.34 ^A
0.1%	6 h		5.09 ± 0.01 ^d	4.95 ± 0.03 ^B	2.65 ± 0.54 ^f	3.07 ± 0.12 ^{AB}	17.04 ± 0.07 ^e	44.15 ± 1.31 ^E
	12 h		5.16 ± 0.00 ^a	5.12 ± 0.02 ^A	4.82 ± 0.03 ^a	2.55 ± 0.03 ^{BC}	47.42 ± 0.42 ^{cd}	47.78 ± 1.04 ^{CD}
	24 h		5.15 ± 0.01 ^b	5.13 ± 0.02 ^A	2.41 ± 0.09 ^f	1.93 ± 0.50 ^D	48.86 ± 0.58 ^{ab}	48.27 ± 0.18 ^{CD}
0.2%	6 h		5.14 ± 0.00 ^b	4.95 ± 0.03 ^B	2.85 ± 0.12 ^{cf}	3.45 ± 0.45 ^A	17.86 ± 0.66 ^e	44.20 ± 0.39 ^E
	12 h		5.11 ± 0.00 ^c	5.17 ± 0.02 ^A	3.47 ± 0.26 ^{bcd}	2.33 ± 0.52 ^{CD}	49.43 ± 0.11 ^a	49.59 ± 0.62 ^{BC}
	24 h		5.13 ± 0.00 ^b	5.15 ± 0.01 ^A	3.77 ± 0.41 ^{bc}	2.15 ± 0.21 ^{CD}	47.99 ± 0.50 ^{bc}	50.87 ± 1.67 ^B
Two-way ANOVA								
Salt concentration (C)			Ns	Ns	*	**	**	***
Soaking time (ST)			***	**	***	***	***	***
C*ST			***	Ns	***	—	*	Ns
SE±			0.004	0.028	0.203	0.191	0.400	0.765
LSD			0.013	0.083	0.600	0.565	1.181	2.258

Data were evaluated using a two-way ANOVA, with soaking time and citric acid concentration as factors for each date-seed cultivar. Capital caps letters indicate significant differences among the Sagae cultivar, whereas lower caps letters indicate significant differences among the Khalas cultivar. Within the column means with identical letters indicate that the values do not differ significantly at $p < 0.05$, according to LSD. Asterisks (*) indicate significantly influential factors as follows: ns, not significant; **, significant at $p \leq 0.01$; ***, significant at $p \leq 0.001$ level. Each value represents the average of the three replicates.

respectively. For Sagae date seeds, the axes contribution of the PC1 and PC2 appeared as 36.71 and 29.06%, with overall variability (65.77%). In comparison, 40.26% (PC1) and 24.30% (PC2) were observed, with an overall variability of 64.56% in Khalas date seeds. The biplot showed a positive association between bioactive compounds and antioxidant activity of germinated seeds from the Sagae cultivar after soaking in a 0.1% citric acid solution for 6, 12, and 24 h, in the second and third quadrants of the plot. However, soaking seeds in a 0.2% citric acid solution for 6 h shows a stronger positive interaction, as indicated by a variable at the $<90^\circ$ level (Figure 2A). Similarly, the pretreatment soaking for 6 h in 0.1% citric acid solution showed in the third quadrants plot the most positive interaction with the variables in germinated Khalas seeds (Figure 2B). Accordingly, the PCA pilot displayed that prior germination, soaking of Sagae and Khalas date seeds for 6 h in 0.2% and 0.1% acetic acid solution could enhance its bioactive compounds, antioxidant activity and colour parameters.

The PLS validation model (Figures 2B, 3A) showed an interactive effect of the pretreatment process (x variables) on the evaluated parameters for germinated date seeds of Sagae and Khalas, respectively. Interestingly, the PLS displayed that the soaking of Sagae seeds in 0.2% citric acid solution for 6 h, and the soaking of Khalas date

seeds for 6 h in 0.1% citric acid solution are the most valid pretreatment conditions for producing germinated date seeds with high levels of bioactive and antioxidant compounds, which might be considered for further application in food manufacturing.

3.5 Bioactive compounds and antioxidant activity of fortified wheat flour

The composition of the main bioactive components in optimally germinated date seed flour was determined before fortification. For both Khalas and Sagae cultivars, germinated date seed flour showed high levels of γ -aminobutyric acid (GABA), carotenoids, total phenolic content (TPC), total flavonoid content (TFC), and strong antioxidant activities as measured by ABTS, DPPH, and FRAP.

Figures 4A,B describes the content of the γ -aminobutyric acid (GABA) and carotenoids of wheat flour fortified with germinated date flour of Khalas and Sagae cultivars. The GABA content of the wheat flour was found to be 6.04 mg/g. Replacing wheat flour with germinated date flour significantly increases GABA content. As the substitution levels increased, the GABA content also increased, particularly at 10%, where the values for the Khalas and Sagae blends were 8.1 and 8.2 mg/g, respectively (Figure 4A). The content of

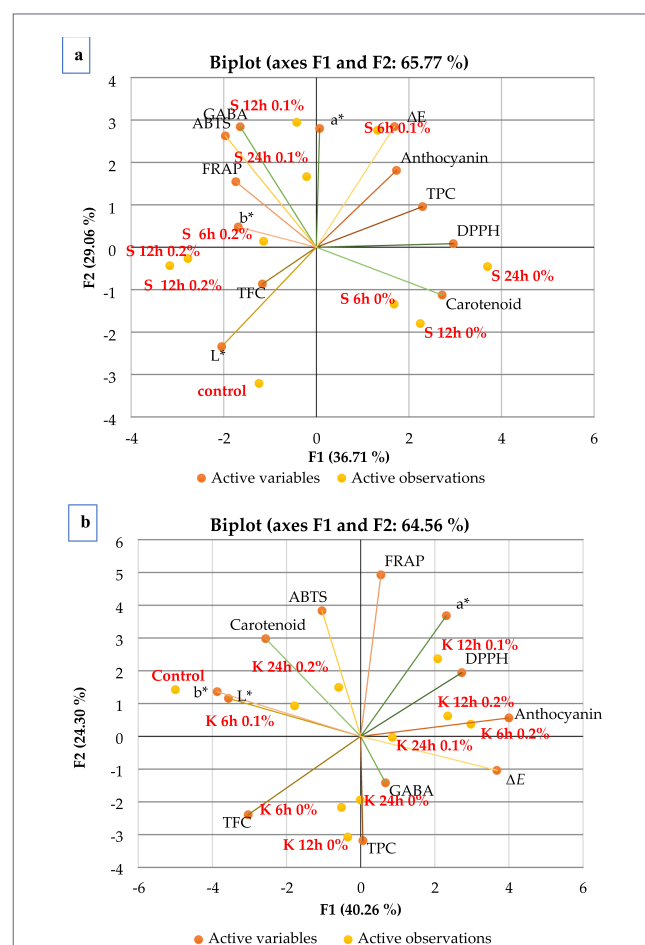
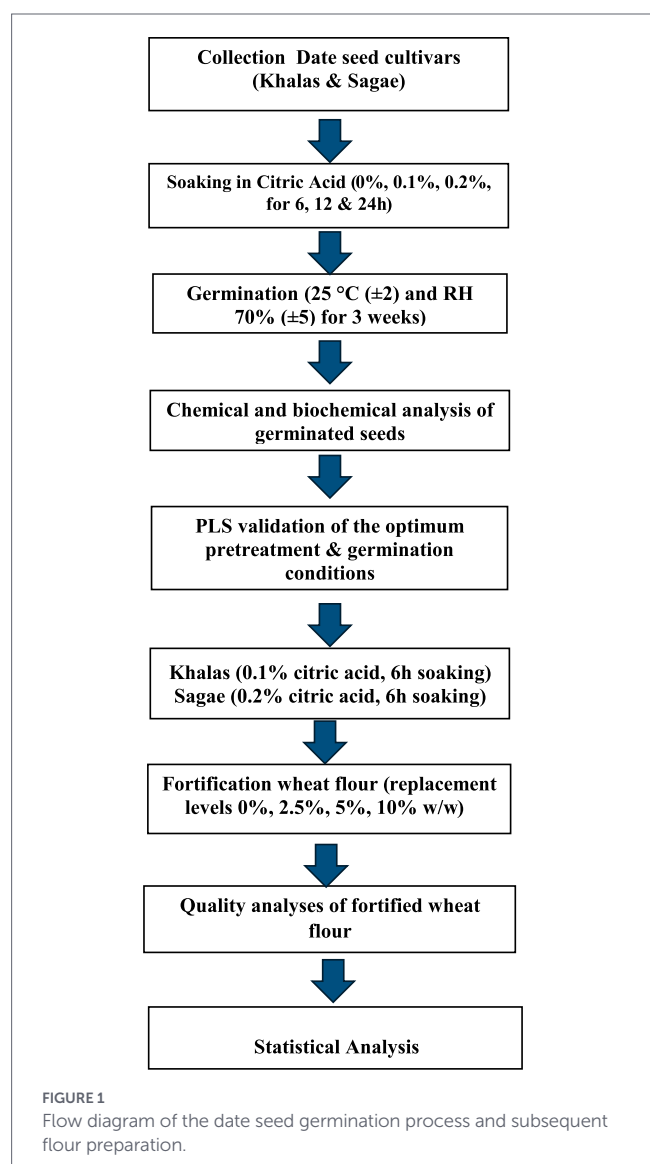


FIGURE 2

The principal components analysis of phytochemical compounds and antioxidant activities of the germinated date seeds of Sagae (a) and Khalas (b) affected by citric acid concentrations and soaking times.

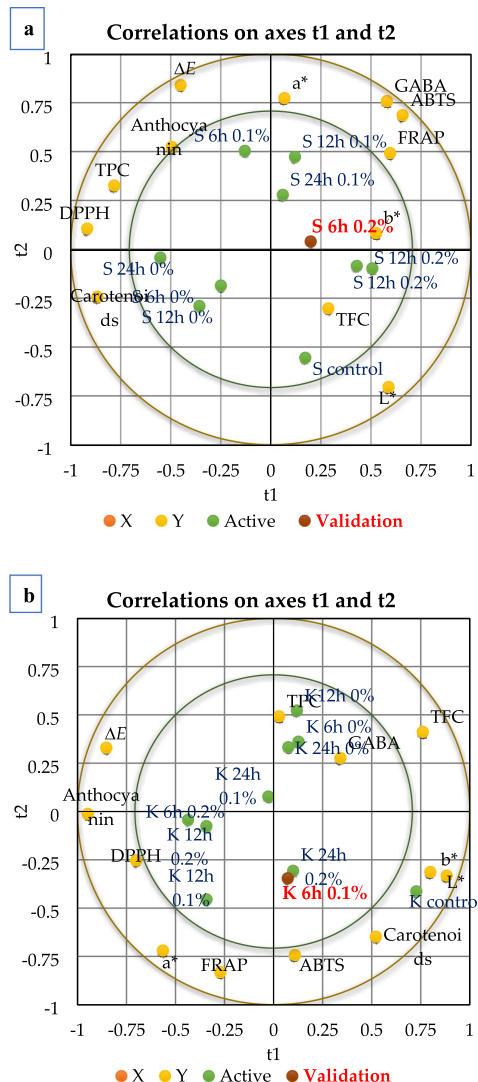


FIGURE 3
Partial least regression validation model of phytochemical compounds and antioxidant activities of the germinated date seeds of Sagae (a) and Khalas (b) affected by citric acid concentrations and soaking times.

carotene of wheat flour alone or repeated with germinated date seed flour is illustrated in Figure 4B. The carotenoid content of the wheat flour was found to be 0.77 mg β -carotene/g. Similar to GABA, the replacement caused a significant increase in carotenoid content. It increased to 1.174, 1.559, and 1.720 mg β -carotene/g with the replacement of germinated Sagae date seeds. Whereas replacing wheat flour with germinated Khalas date seeds at levels of 2.5, 5.0, and 10% significantly increased the carotene content to 1.1174, 1.559, and 1.720 mg β -carotene/g, respectively.

Figure 5A shows the total phenolic content (TPC) for control and fortified wheat flour. The phenolic content was found to be 9.063 mg GAE/g in wheat flour (0% replacement). Replacement of wheat flour with germinated date seed flour obtained from both cultivars, Sagae and Khalas, at levels up to 5% did not significantly affect TPC. However, replacement at 10% sharply increased TPC to 31.1 and 28.8 mg GAE/g for Sagae and Khalas replacement, respectively. Likewise, the total flavonoid content (TFC) follows the same pattern as the TPC (Figure 5B). The Sagae blends showed a bigger

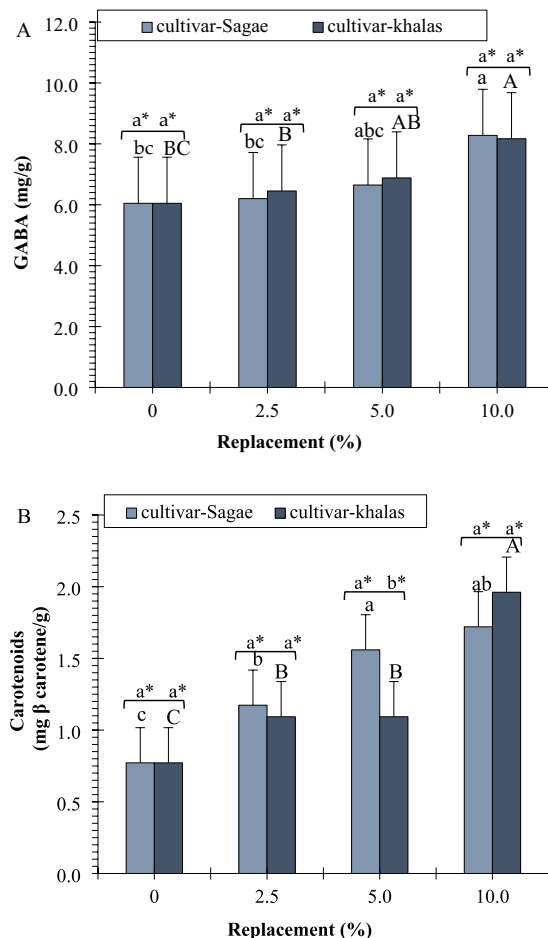
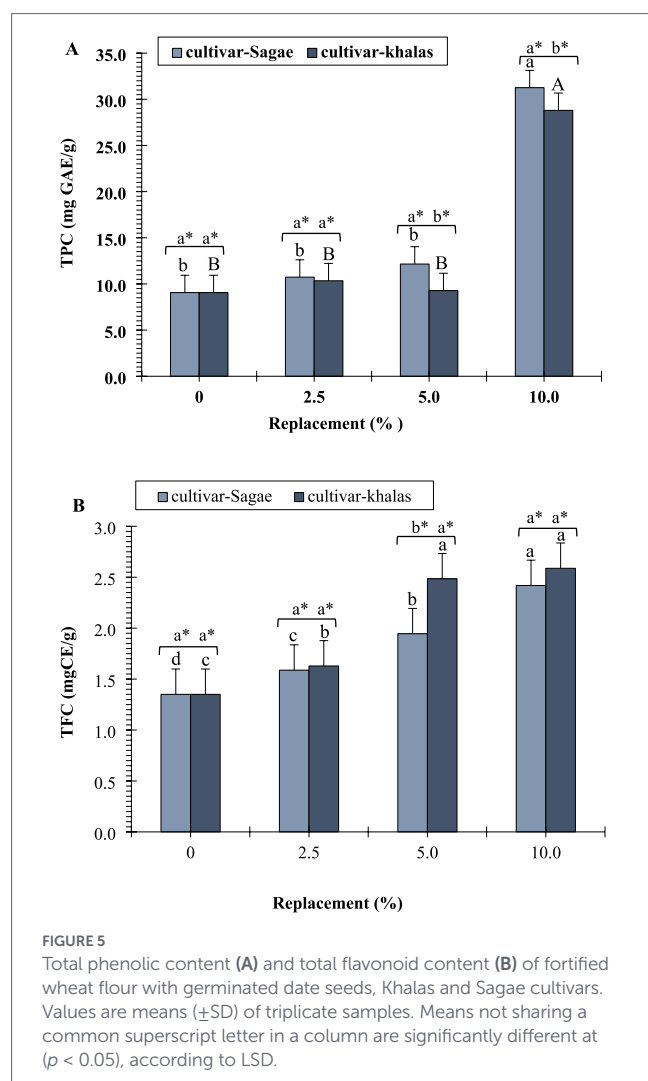


FIGURE 4
GABA content (A) and total carotenoids (B) of fortified wheat flour with germinated date seeds, Khalas and Sagae cultivars. Values are means ($\pm$ SD) of triplicate samples. Means not sharing a common superscript letter in a column are significantly different at ($p < 0.05$), according to LSD.

increase, especially at 10% fortification, when TPC reached 31.2 mg GAE/g.

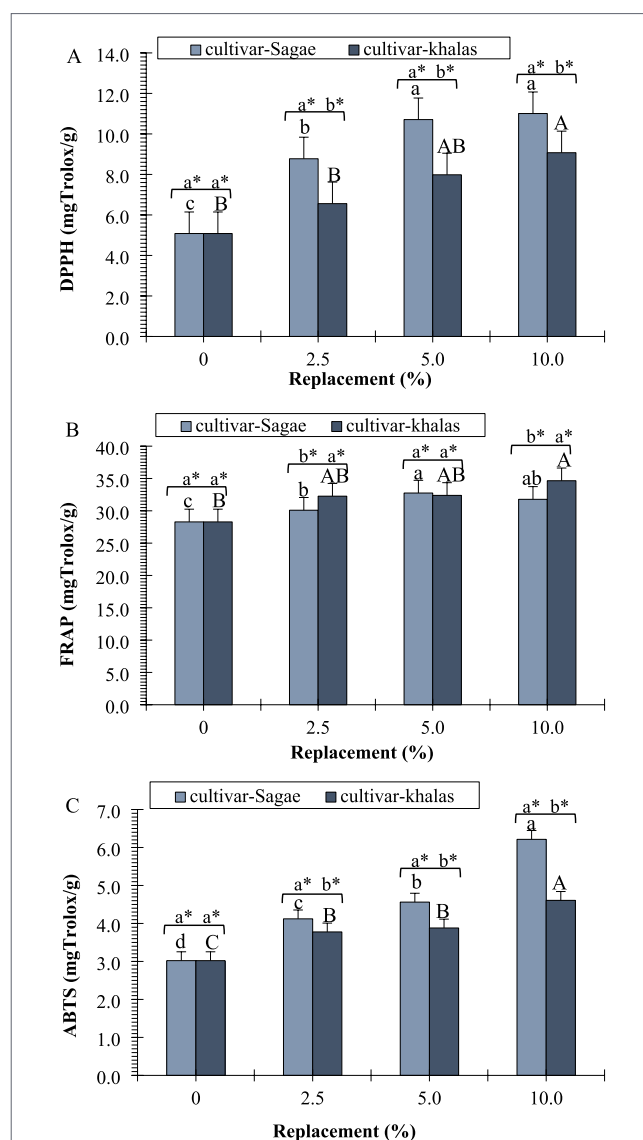
The ABTS, DPPH, and FRAP assays all showed the same trend in antioxidant activity, with the highest values observed in the 10% Sagae blends. This indicates that adding germinated date seed flour significantly improved the antioxidant potential (Figure 6).

Sharma and Gujral (2014), determined that the total phenolic content (TPC) of wheat flour was 2062 mg FAE/g. The addition of barley flour at substitution levels of 28, 56, and 84 g/100 g of wheat flour resulted in significant increases in TPC of 26.5, 49.5, and 57.1%, respectively. Barley flour, known for having more phenolic compounds than wheat, contributed to the increase in these numbers. Additionally, they reported that the total flavonoid content of wheat flour increased to 1,275, 1,603, and 1,941 mg CE/g when supplemented with barley flour. Moreover, the antioxidant activity, measured by DPPH radical scavenging activity, increased from 12.3% in wheat flour to 16.4, 19.6, and 22.6% in the corresponding barley-substituted blends. These results demonstrate that incorporating germinated date seed flour or barley flour into wheat flour significantly enhances the bioactive compound profile, total phenolic and flavonoid contents, and antioxidant potential of the resulting blends. The gradual increase in health and functional benefits was observed with higher substitution levels.



These results suggest that the observed increases in GABA, carotenoids, phenolic and flavonoid contents, and antioxidant activities are mainly attributable to the compositional contribution of germinated date seed flour. These findings agree with [Sharma and Gujral \(2014\)](#), who reported that supplementing wheat flour with barley flour, which is also rich in phenolic compounds, resulted in significant increases in total phenolic and flavonoid contents, as well as antioxidant activity.

Similarly, the gradual increase in health and functional benefits observed with higher substitution levels in this study is consistent with their findings, further supporting the use of functional ingredients, such as germinated date seed flour, to enhance the nutritional profile of wheat-based products. Moreover, since bioactive and antioxidant compounds such as GABA, carotenoids, and phenolic compounds play a vital role as health-promoting compounds with antioxidant action, increasing their levels in fortified wheat flour enhances the total antioxidant capacity of the flour matrix and improves oxidative stability during storage. Also, increasing such compounds provides significant techno-functional benefits for the fortified flour, including improved high-temperature stability and preservation of antioxidant properties before and after baking and frying. Moreover, the food industry is improving its products in search of natural preservation alternatives ([Chen and Xia, 2024](#)).



4 Conclusion

In summary, the current study demonstrates that pre-treating date seeds with citric acid at different concentrations (0.1% and 0.2%) and soaking times (6, 12, and 24 h) before germination significantly increases their bioactive compound content and antioxidant activity. The synergistic effect of these factors was demonstrated by notable elevations in phenolic compounds, flavonoids, GABA, anthocyanins, and antioxidant capacity, especially under optimal conditions. The PLS analysis showed that the most valid pretreatments were 0.1% citric acid for 6 h for the Khalas cultivar and 0.2% citric acid for 6 h for the Sagae cultivar. Additionally, this study found that combining germinated date seed flour with wheat flour significantly enhances its nutritional value, particularly in terms of bioactive compounds and antioxidant activity. These findings suggest that germinated date seed flour is a promising, underutilised ingredient for improving the health

benefits of wheat-based food products, particularly in regions with abundant date production. However, further studies are also required to evaluate the impact of other treatments, either with these cultivar seeds or with other cultivars. Moreover, further study is recommended to evaluate the techno-functional properties and sensory attributes of the fortified flour, providing insights into utilising this byproduct ingredient to improve the nutritional and rheological quality of wheat-based products.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

LA: Data curation, Formal analysis, Methodology, Writing – original draft. SH: Investigation, Supervision, Writing – original draft. MA: Investigation, Supervision, Writing – original draft. AH: Conceptualization, Data curation, Investigation, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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