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ORIGINAL PAPER

Nutritional components and antioxidant activities of *Firmiana platanifolia* seeds in response to different germination times*

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Abstract

Germination can be used to enhance nutritional value and health functions of edible seeds. Sprouts are considered healthier than non-germinated seeds because they are richer in the basic nutritional components (protein, amino acid, carbohydrates, and minerals) and also contain more bioactive components. The present study is the first to dynamically analyze the germination properties regarding morphological characteristics, nutrient compounds, bioactive components and antioxidant activities of *Firmiana platanifolia* (*F. platanifolia*) seeds. The results showed that the appearance, color and morphology of *F. platanifolia* seeds and sprouts at different germination times showed considerable differences. With the increasing germination time, the moisture content, sucrose, fructose, total flavonoid content and DPPH significantly increased, and reached the peak at 8 d of germination, almost 2.38, 5.76, 4.02, 7.97 and 8.91 times higher than those of at 0 d, respectively. The soluble protein, total phenolic, ABTS and ferric reducing antioxidant power (FRAP) increased first and then decreased as the germination time increased, and were overall higher than those of at the initial concentration. However, total starch content and mineral elements decreased as germination progressed. Additionally, the total flavonoid content showed a strongly positive correlation with DPPH ($p<0.01$), whereas the total phenolic and total flavonoid content showed a similar, significant and positive correlation with ABTS and ferrous ion chelating activity (FICA, $p<0.05$). Therefore, seed germination significantly increased phytochemical contents and antioxidant activity. The current research results will help to reveal the dynamic changes in nutrient content and antioxidant activity of *F. platanifolia* seeds during the germination process, and provide reference data for further studying the development and utilization of germinated seeds.

Keywords: *Firmiana platanifolia*, germinated, nutritional component, element content, antioxidant activity

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INTRODUCTION

The seed is a critical organ in the life cycle of higher plants needed for their survival (Zaynab et al. 2021). It may survive the period between seed maturation and the establishment of the next generation as a seedling after germination (Bentsink, Koornneef 2008). Germination, also known as sprouting, refers to the process where seeds with vital signs transform from transiently dormant to the dynamic physiological state (Aparicio-García et al. 2021). As a start and core event in the life cycle of plants, seed germination establishes the basis for the next generation, which is the result of the synergistic action of all physiological and biochemical systems inside seeds (Zhang et al. 2017). Germination is a complex process, in which significant changes in the biochemical, nutritional, and sensory characteristics occur due to the activation of dormant enzymes, and germination is also one of the most common and effective processes to improve the nutritional quality of seeds (Zhang et al. 2015). Therefore, when compared to dormant seeds, germinated seeds or sprouts had a higher bioavailability and bioaccessibility of nutrients, minerals and vitamins (Pająk et al. 2019).

Recently, the global prevalence of various diseases has been on a gradual rise, in part due to unhealthy eating habits (Aloo et al. 2021). Free radical reactions may lead to the formation of reactive oxygen species (ROS), which play critical roles in various human diseases (Yan et al. 2015). In food, free radicals not only lead to the deterioration of quality attributes, such as flavor, aroma, texture, and color, but also to the loss of nutritive value (Kou et al. 2013). In an organism, free radicals are involved in a variety of diseases and can cause the damage of protein, mutation of DNA, oxidation in cell membrane phospholipids, and consequently may adversely affect immune functions (Li et al. 2015). Meanwhile, the continuously growing demand for promising dietary antioxidant sources has triggered the search for newer, economical, nutritional and multifunctional sources possessing free radical scavenging potential (Liu et al., 2022). Nevertheless, the nutritional properties and bioactive compositions (such as amino acids, sugars and phenolic compounds) of natural dietary plants play an important role in human nutrition and health as potential sources of functional foods and nutraceuticals (Abeysinghe et al. 2007, Deepa et al. 2007). Germination process is an essential tool to improve the nutritional value and sensorial quality of food in the food industry. Many studies have demonstrated that dynamic changes during germination may improve the nutritional and nutraceutical values of seeds by causing desirable changes in the chemical composition and the availability of vitamins and minerals, increasing the levels of free amino acids, dietary fiber, and other components (Perales-Sánchez et al. 2014, Wang et al. 2015). Moreover, germination not only reduces or eliminates antinutritional and indigestible compounds, but also improves the functionality of the seeds in the bioactive compounds and asso-

ciated antioxidant activity (Chu et al. 2019). Therefore, germinated edible seeds and sprouts can be consumed as functional foods for the prevention of certain chronic diseases.

Firmiana platanifolia is a deciduous tree of the family Sterculiaceae originating from China. It is a tall, deciduous tree that has been used as an ornamental and medicinal plant for roadsides and courtyards in China, Korea, Europe and the USA (Woo et al. 2016). Many active components have been found in the stem, leaf, flower and seed of *F. platanifolia*. *F. platanifolia* seeds, which have been used as a traditional Chinese herb for promoting digestion and treating stomachache, are sweet in taste, neutral in reaction, and nontoxic (Sun, Li 2016). Furthermore, seeds have a very high nutritional value, containing large amounts of protein, fat, polysaccharide and minerals elements (Sun et al. 2018). In particular, the oil content of *F. platanifolia* seeds was 27.8 ± 0.3%, comprised mainly of fatty acids (palmitic acid, 17.4%; oleic acid, 22.2%; linoleic acid, 30.2%), and was therefore a new potential, promising and undeveloped renewable resource (Sun, Li 2016). So far, little information about the phytochemical substances in *F. platanifolia* seeds has been reported, let alone changes in nutritional components and antioxidant activities during the germination periods. Consequently, in this study, a dynamic analysis of nutritional components and antioxidant activities of seed and sprout extracts from *F. platanifolia* was performed at different germination times. The contents of moisture, soluble protein, total amino acids, total starch, sucrose, fructose, total phenolic, and total flavonoid of *F. platanifolia* seeds germinated for 0, 2, 4, 6, and 8 d were measured. Meanwhile, the antioxidant activities of *F. platanifolia* seeds during germination were analyzed according to DPPH radical scavenging activity, ABTS radical scavenging activity, the ferric reducing antioxidant power (FRAP), and the ferrous ion chelating activity (FICA) methods. These results will present information to further develop and utilize germinated seeds of *F. platanifolia* as a potential functional food for human consumption.

MATERIAL AND METHODS

Materials and reagents

F. platanifolia seeds were collected in Chengdu Campus of Sichuan Agricultural University, Chengdu, China, in October 2021. They were shelled, sorted, placed in labelled net bags, and then stored in ventilated, dry and cool places for subsequent seed germination. Ascorbic acid, ninhydrin, anthrone, ammonium molybdate, Folin-Ciocalteu reagent, potassium persulfate, ferrozine, Coomassie brilliant blue and bovine serum albumin were purchased from Tauto Biotech (Shanghai, China). Flavonoids and phenolic acid standards were purchased from Tauto Biotech (Shanghai, China) and Aladdin Chemistry Co., Ltd. 1,1-diphenyl-2-picrylhydrazyl (DPPH),

2,2′-azino-bis (3-ethylbenzothiazoline-6-sul-phonic acid) (ABTS), 2,4,6-tri (2-pirydyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, USA). All other chemicals and solvents (anhydrous ethanol, phosphate buffer, glucose, concentrated sulfuric acid, hydrogen peroxide, potassium dichromate, ferrous sulfate, sodium hydroxide, sodium carbonate, sodium nitrite, aluminum nitrate, and ferrous chloride) used were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Seed germination

The germination process was carried out according to the method published by Pająk et al. (2019). Healthy plump seeds were sterilized for 30 s by immersion in ethanol (96%) and washed with deionized water 3 times. Then, the seeds were bathed at 35°C for 24 h. After pouring off the soaking water, the seeds were evenly spread on sterile trays with four layers of gauze and were sprayed twice a day for 30 s using deionized water. Subsequently, the germination process of the seeds was carried out at temperature in the range of 28 ± 2°C 12/12 h day/night for 8 d. The seeds were observed every two days, and morphological characteristics of seeds were recorded on the day. The seeds were individually taken at 0, 2, 4, 6 and 8 d, and the endosperm was isolated without the seed coat and stored until further analyses. Three replications were made for each germination condition. At least 100 seeds were germinated for each time point for each biological replication.

Determination of nutrient compositions

The nutrient compositions and antioxidant activity of sprouts and seeds were measured separately. The moisture content was determined by using the oven-dry method (Le et al. 2021). Briefly, 2.0 g of *F. platanifolia* sprouts were dried in an oven at 105°C to constant weight. The measurements were expressed as the percentage of dry weight. The soluble protein content was measured by the Bradford's method using bovine serum albumin as the standard (Song, Tang 2016). The total free amino acids content was determined by the UV spectrophotometry (Ye et al. 2020). The total starch content was measured using the enzyme hydrolysis method (Rayo-Mendez et al. 2019). The fructose and sucrose contents were calculated using the vitriolic acid-phenol method (Chen et al. 2022).

Determination of micro- and macroelements

Mineral composition of *F. platanifolia* sprouts was determined by atomic absorption spectrophotometry (Bhinder et al. 2020). The germinated seeds at different stages were digested in a digestion flask with sulfuric acid (10 mL), followed by the addition of hydrogen peroxide (5 mL). Subsequently, these seeds were heated in a heating block at 420°C for 60 min, and hydrogen peroxide (5 mL) was added again. Then, the digestive solution was boiled for a further 60 min until the solution was clear. Next, the digestive solution

was cooled by cautiously adding 50 mL of deionized water. The potassium dichromate oxidation method, semimicro-Kjeldahl method and Mo-Sb colorimetric method were used to determine the C, N and P content, respectively (Hu et al. 2019). The contents of calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) were analyzed in an atomic absorption spectrophotometer calibrated with standard mineral solutions (Le et al. 2021).

Determination of the content of total phenolics and flavonoids

The content of phenolic and flavonoid compounds was determined in fresh matter of sprouts and seeds. The procedure of methanolic extraction was carried out according to the method described in Yu et al. (2018). The filtrate was used for determination of total phenolic content (TPC) and total flavonoid content (TFC). Total phenolic content was determined by the Folin-Ciocalteu method as reported in Pająk et al. (2017). Total flavonoid content (TFC) was measured by the aluminum chloride colorimetric method described by Roberts, Moreau (2016).

Determination of antioxidative activity

The DPPH radical scavenging activity was measured following the method of Pająk et al. (2014). Briefly, 0.1 mL extract of samples with different germination time was mixed with 3.9 mL of 0.1 mM DPPH radical in methanol. The mixture was shaken immediately and then incubated in the dark for 60 min. The absorbance was then recorded at 517.0 nm in an 8453 UV/Visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The DPPH radical scavenging activity in the extracts was expressed as g of Trolox equivalents per kg of dry mass of seeds and sprouts.

Determination of the ABTS radical scavenging activity of methanolic extracts of seeds and sprouts was carried out according to the methods of Pająk et al. (2017). ABTS cation radicals were generated by the reaction of 7 mM ABTS stock solution with 2.45 mM potassium persulfate. The mixture was incubated in the dark for 12-16 h. Then, the ABTS solution was diluted with 0.01 M phosphate buffer saline (pH 7.4) to obtain the absorbance of 734.0 nm of 0.70±0.02. Next, 0.1 mL of sample from each of the various germination times was mixed with 0.9 mL of diluted ABTS solution. The reaction mixture was incubated for 25 min at 30°C. Finally, the absorbance was recorded at 734.0 nm. The ABTS radical scavenging activity in the extracts was expressed as g of Trolox equivalents per kg of dry mass of seeds and sprouts.

The FRAP of the methanolic extracts was measured according to the method of Zambrano et al. (2020). Briefly, the FRAP solution was prepared by mixing sodium acetate buffer (pH 3.6), 10 mM TPTZ and 20 mM $FeCl_3$ $6H_2O$ at a ratio of 10:1:1. Next, 1.5 mL sample solution with different germination time was added to 4 mL of fresh FRAP solution, and then the mixture was incubated at 37°C for 30 min in the dark. The absorbance was measured

at 593.0 nm. The FRAP was expressed as mmol of Fe^{2+} per 100 g of dry mass of seeds and sprouts.

The ferrous ion chelating activity (FICA) was investigated according to the reported method (Thambiraj et al. 2018). Five samples were prepared with different germination time. The samples (1.0 mL) were mixed with 0.1 mL of 2 mM $FeCl_2$, 0.15 mL of 5 mM ferrozine, and 0.55 mL of methanol. Subsequently, the mixture was vortexed and incubated at room temperature for 10 min. The absorbance of the reaction mixture was measured at 562.0 nm. EDTA solution was used as a positive control.

Statistical analysis

The values of all indices determined in the experiment were obtained in triplicate and expressed as a mean ± standard deviation (SD). MS Office Excel 2019 and SPSS software (version 20.0, Chicago, USA) were used for data statistics and analysis. One-way ANOVA and LSD (least significant difference) methods were used to test the significance of differences between different germinated stages ($p<0.05$). The Pearson's linear correlations coefficients between selected parameters were also calculated in SPSS 20.0 software. Origin 8.5 served to plot diagrams.

RESULTS

Changes in morphological characteristics

Figure 1 shows the appearance, color and morphological characteristics of *F. platanifolia* seeds and sprouts at different germination times.

Fig. 1. Changes in morphological characteristics of the transverse and longitudinal section profile at different germination times: *a* – transverse profile, *b* – longitudinal section profile

F. platanifolia are dicotyledonous plants, with seeds composed of the embryo, seed coat and endosperm. When seeds were saturated with water for 2 d, the radicle began to elongate and gradually broke through seed coat. After germination for 2, 4, 6 and 8 d, the radicle length of *F. platanifolia* increased to 1.8 mm, 3.9 mm, 10.9 mm and 25.1 mm, respectively. As the radicle elongates continuously, the kernel and cotyledon began to develop. Meanwhile, significant variations were observed in the shape and weight of the endosperm at different germination times. Although initially spherical at the non-germinated period, the shape of endosperm became flat as germination time, and the color varied from gray to white. With prolongation of the germination time, the longitudinal length of the endosperm in the longitudinal section increased gradually, reaching the maximum value of 13.8 mm on the 8th day. Interestingly, the transverse length of the endosperm in the transverse section reached the maximum value of 9.4 mm on the 4th day, and then decreased gradually. Similarly, the color of cotyledon varied, from milky white to light green, and the cotyledon grew with the endosperm.

Changes in relative water content, soluble protein and amino acids content

There were significant differences in the moisture content at different germination times (Figure 2*a* and Table 1). The non-germinated seeds had a low initial water content. With the increase of germinating time, the moisture content increased gradually. The moisture content ranged from 33.0% to 78.8%, with the highest and the lowest being for 8 d and 0 d, respectively. After germination for 2, 4, 6, and 8 d, the moisture content of sprouts increased to 55.3%, 63.3%, 68.8% and 78.8%, respectively. The highest increase was observed for 2 day- and the lowest increase was for 6 day germination.

The content of soluble protein varied significantly ($p<0.05$) in sprouts with different germination times (Table 1). According to Figure 2*b*, the soluble protein content was 252.1, 293.5, 340.4, 426.7, and 262.5 g kg^{-1} at 0, 2, 4, 6 and 8 d of germination, respectively. The soluble protein content increased first and then decreased as the germination time increased, and the maximum was obtained for 6 d, showing that germination could significantly increase the soluble protein content of *F. platanifolia*.

As shown in Figure 2c and Table 1, the results suggested that a different germination time causes significant differences in the total amino acid content ($p<0.05$). The total amino acid content and moisture content showed the same trend. The total amino acid content in germinated *F. platanifolia* seeds increased with an increase in germination time. Non-germinated *F. platanifolia* seeds also contained a small amount of total amino acids, and their content increased during germination. It ranged from 0.16 to 7.38 g kg^{-1}, with the highest and the lowest being for 8 d and 0 d, respectively.

Table 1

One-way ANOVA of nutritional components, mineral element content and antioxidant capacity at different germination times

Source of variation	Germination time		
	df	*F*	*P*
Moisture content	4	117.993	<0.001
Soluble protein	4	40.992	<0.001
Total amino acids	4	322.423	<0.001
Total starch	4	28.424	<0.001
Sucrose content	4	208.933	<0.001
Fructose content	4	45.390	<0.001
C content	4	78.210	<0.001
N content	4	48.795	<0.001
P content	4	154.548	<0.001
K content	4	26.158	<0.001
Ca content	4	2.968	0.074
Mg content	4	13.994	<0.001
Na content	4	5.864	0.011
Total phenolic	4	23.970	<0.001
Total flavonoid	4	62.337	<0.001
DPPH	4	78.458	<0.001
ABTS	4	41.883	<0.001
FRAP	4	1.050	0.429
FICA	4	9.380	0.002

FRAP – ferric reducing antioxidant power, FICA – ferrous ion chelating activity

Changes in total starch, sucrose and fructose contents

We found significant differences in total starch, fructose and sucrose content between various germination times (Table 1). Results of total starch (a), fructose (b) and sucrose content (c) of *F. platanifolia* seed at different germination times are shown in Figure 3. It was observed that due to germination, the total starch content considerably decreased by 38.0% from 0 to 6 d, reaching the minimum at 6 d of germination, at around 7.8 g kg^{-1}, and then increasing to around 8.31 g kg^{-1} for the 8 d germinated seed extracts. Moreover, a similar trend in the fructose and sucrose content of germinated seeds during germination was observed. It was noted that during germination, the fructose and sucrose content increased gradually from 0 to 8 d of germination. *F. platanifolia* sprouts showed an increase in the sucrose and fructose content by 4.8 times and 3.0 times at 8 d of germination, respectively. The maximum increase of the sucrose and fructose content was 124.4% and 72.4% at 8 d of germination, respectively.

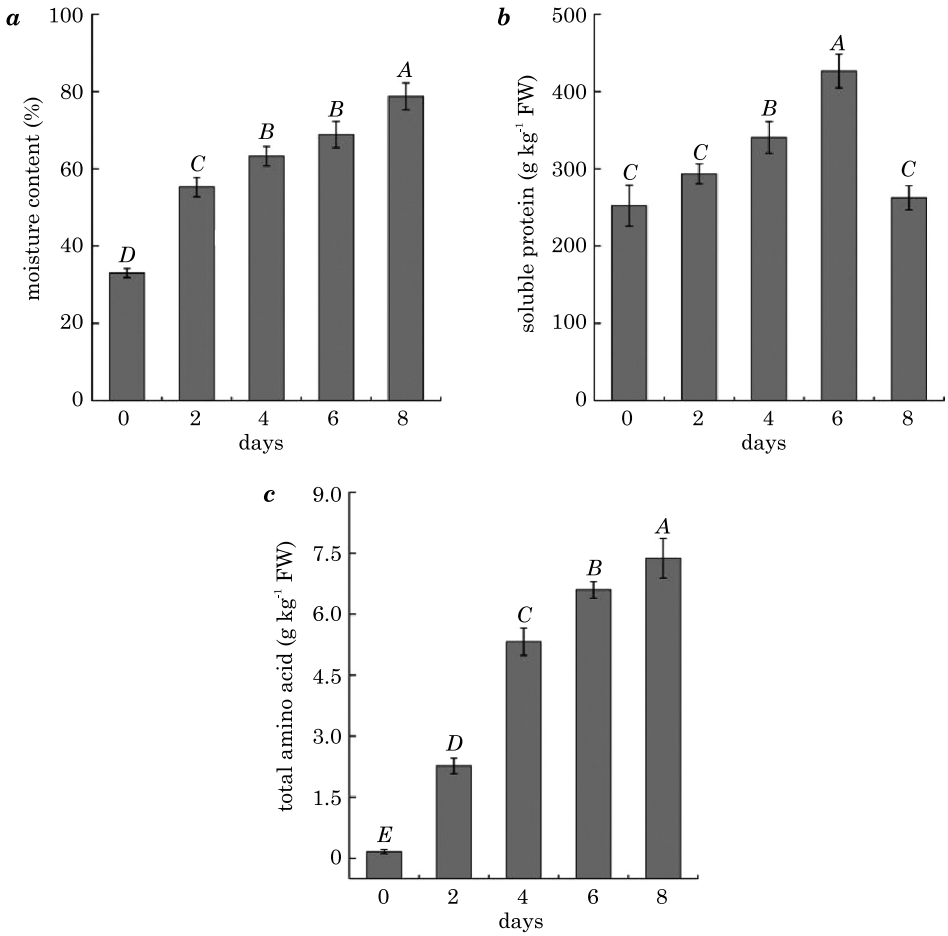

Fig. 2. Changes in moisture, soluble protein and total amino acids of germinated seeds at different germination times: *a* – moisture content, *b* – soluble protein content, *c* – total amino acids, FW – fresh weight, data are shown as means ± SE (n=3). Different uppercase letters indicated significant difference at different germination times (p<0.05)

Changes in macro- and microelements

As shown in Tables 1, the results suggested that the germination time had significant effects on the C, N, P, K, Mg, and Na content (p<0.05), but it had no significant effect on the Ca content in seeds and sprouts (p>0.05). The macro- and microelements content in seeds and sprouts is presented in Table 2. There were differences between the concentrations of all the mineral elements studied at different germination times. The C, N, P, K, Ca, Mg, and Na content ranged from 96.4 to 189.3 g kg^{-1}, 10.64 to 28.83 g kg^{-1}, 0.31 to 4.15 g kg^{-1}, 2.41 to 8.42 g kg^{-1}, 2.89 to 4.50 g kg^{-1}, 1.85 to 4.52 g kg^{-1} and 0.032 to 0.078 g kg^{-1}, respectively. However, the macro-elements showed

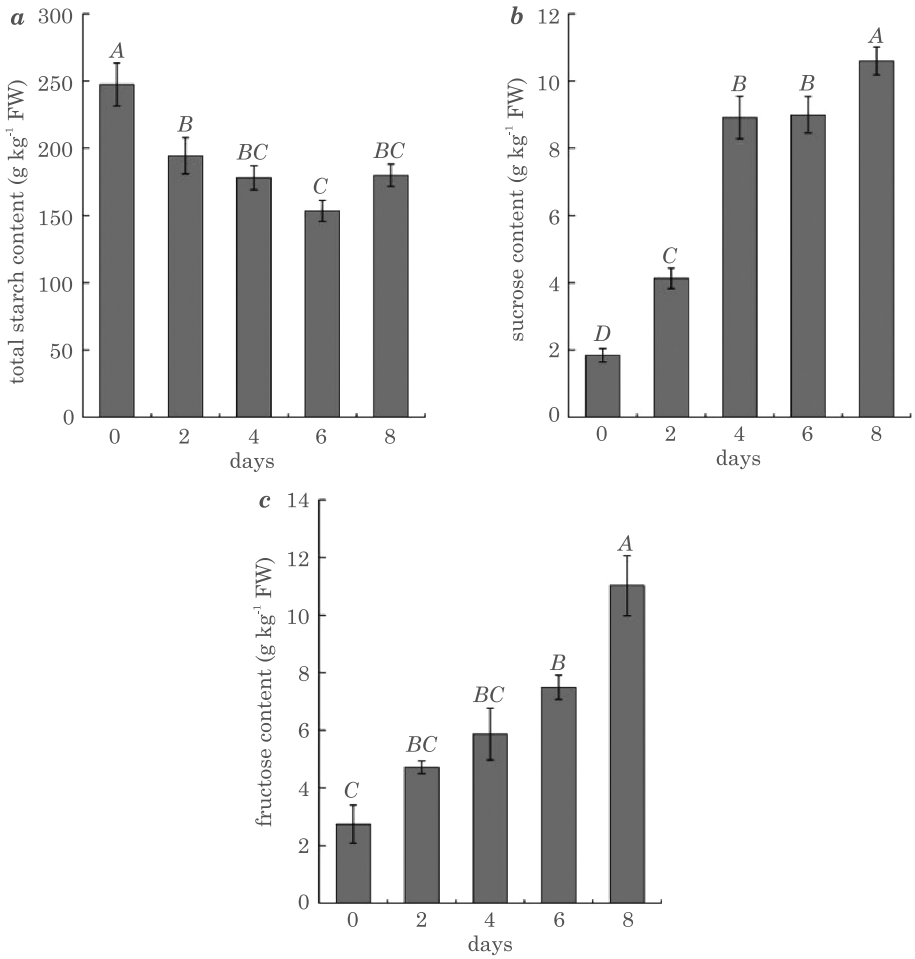

Fig. 3. Changes in sugar content of germinated seeds at different germination times: *a* – total starch content, *b* – sucrose content, *c* – fructose content, FW – fresh weight, data are shown as means ± SE (n=3). Different uppercase letters indicated significant difference at different germination times (p<0.05)

the same trend as the microelement content at different germination times. Meanwhile, content of these mineral elements decreased as the germination time progressed, but further on it decreased significantly. With respect to mineral elements, C was the most abundant mineral in non-germinated seeds, followed by N, K, Mg, Ca, P and Na. Nevertheless, the Ca content was higher than the K and Mg content at 8 d of germination, suggesting that K and Mg are more consumed during germination. Simultaneously, we found that these elements showed no significant differences at 2 d of germination, but a significant difference was found at 6 d of germination. The maximum decrease of C, N, P, K, Ca, Mg and Na content was 49.1%, 63.1%, 92.5%,

Table 2

Changes in macro- and microelements at different germination times

Element (g kg^{-1} DW)	0 d	2 d	4 d	6 d	8 d
C	189.3^{A}±7.0	181.4^{A}±10.8	160.6^{B}±6.9	114.4^{C}±8.44	96.4^{C}±6.2
N	28.83^{A}±2.41	27.12^{A}±1.70	18.83^{B}±2.64	13.34^{C}±1.68	10.64^{C}±1.26
P	4.15^{A}±0.19	3.85^{A}±0.12	2.78^{B}±0.25	1.51^{C}±0.37	0.31^{D}±0.02
K	8.42^{A}±1.19	7.53^{AB}±1.01	5.81^{BC}±0.42	4.11^{CD}±0.81	2.41^{D}±0.44
Ca	4.50±0.83	3.79±0.74	3.41±0.38	3.15±0.44	2.89±0.64
Mg	4.52^{A}±0.63	4.41^{A}±0.52	4.01^{A}±0.72	2.43^{B}±0.54	1.85^{B}±0.36
Na	0.078^{A}±0.018	0.054^{AB}±0.013	0.041^{B}±0.012	0.038^{B}±0.009	0.032^{B}±0.004

DW – dry weight. Values followed by the same letter in the same row are not significantly different according to Tukey's test (α=0.05).

71.4%, 35.8%, 59.1% and 59.0%, respectively, showing that N, P and K were more used rapidly.

Changes in total phenolics and total flavonoids

The total phenolic and total flavonoid content of *F. platanifolia* seeds at different germination times and their significant markers are presented in Table 1 and Figure 4. There were differences between the total phenolic and total flavonoid content at different germination times. The total phenolic content increased first decreased as the germination time increased, and the maximum was obtained for 4 d. The total phenolic content ranged from 1.092

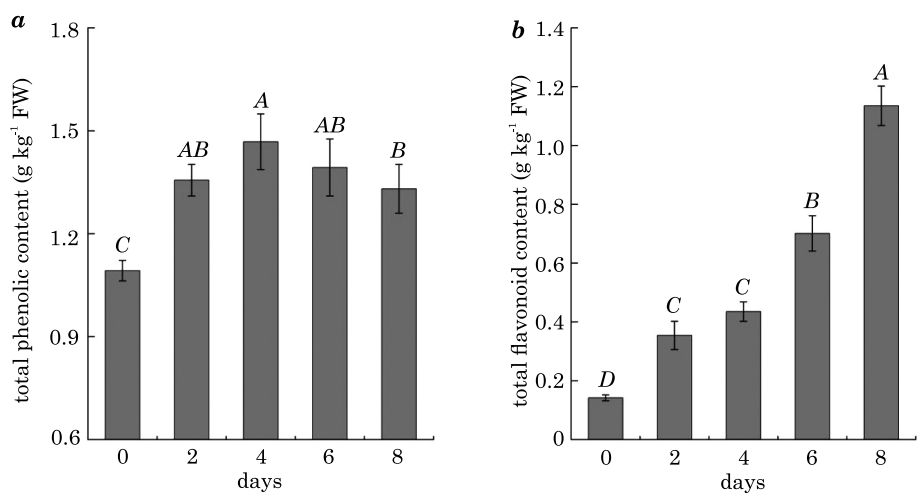

Fig. 4. Changes in total phenolic and total flavonoid content of germinated seeds at different germination times: *a* – total phenolic content, *b* – total flavonoid content, FW – fresh weight, data are shown as means ± SE (n=3). Different uppercase letters indicated significant difference at different germination times (p<0.05)

to 1.468 g kg^{-1} and extensively increased by 34.4% from 0 to 4 d, and then decreased to around 1.331 g kg^{-1} for the 8 d. However, there was a different trend between the total phenolic and total flavonoid content with various germination times. The total flavonoid content increased as the germination time increased, and the total flavonoid content ranged from 0.142 to 1.135 g kg^{-1}, and the highest increase was observed for 8 d and the lowest increase was for 0 d, increasing by 61.9%.

Changes in the antioxidant capacity

The antioxidant activities were evaluated by DPPH radical scavenging ability (a), ABTS radical scavenging ability (b), ferric reducing antioxidant power (FRAP, c) and ferrous ion chelating activity (FICA, d) – Figure 5. There were significant differences in the DPPH radical scavenging ability, ABTS radical scavenging ability and ferrous ion chelating activity at different germination times ($p<0.05$), but there was no significant difference in the ferric reducing antioxidant power ($p>0.05$). All the results revealed a similar tendency with some divergences, irrespective of the methods used in this study (such as DPPH, ABTS, FRAP and FICA) and their different

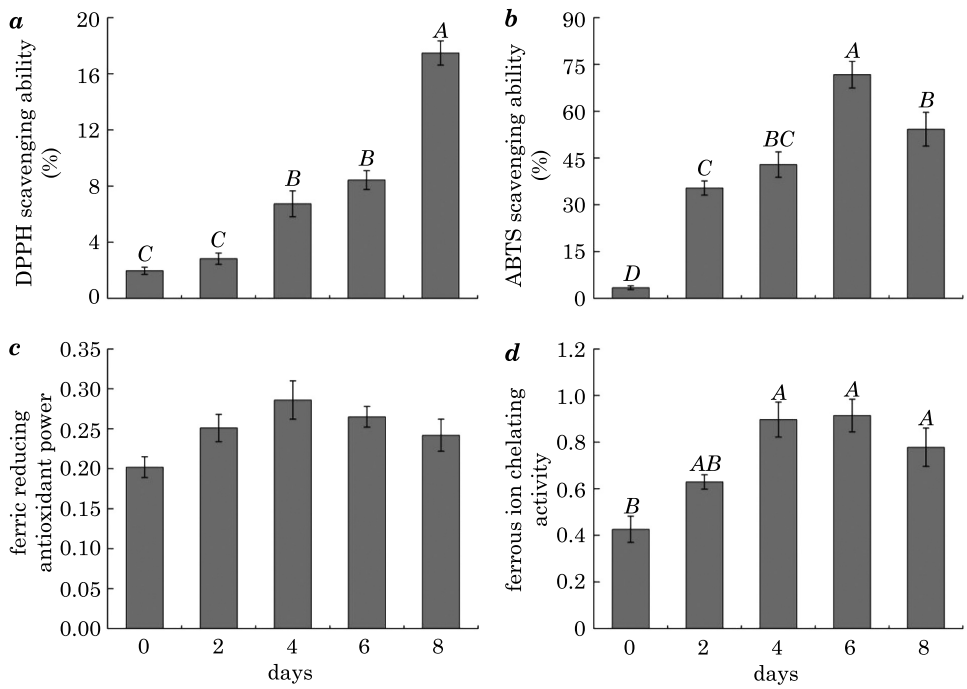

Fig. 5. Changes in antioxidant activities of germinated seeds at different germination times: *a* – DPPH radical scavenging ability, *b* – ABTS radical scavenging ability, *c* – ferric reducing antioxidant power (FRAP), *d* – ferrous ion chelating activity (FICA), data are shown as means ± SE ($n=3$). Different uppercase letters indicated significant difference at different germination times ($p<0.05$)

reaction mechanisms. The ABTS, FRAP and FICA increased first and then decreased as the germination time increased, but the DPPH increased as the germination time increased. Interestingly, the DPPH, ABTS, FRAP and FICA of non-germinated seeds were significantly lower than of sprouted seeds during the whole germination process. In particular, the ABTS and FICA significantly increased from 0 to 6 d, then decreased from 6 to 8 d, and the maximum value was observed for 6 d. In contrast, the DPPH significantly increased from 0 to 8 d, and the maximum value was observed for 8 d, increasing by 7.9 times higher than those of in the controls.

Correlation analysis of nutrients and antioxidant activity

The results of a correlation analysis between the nutrient compounds and antioxidant activity are shown in Table 3. High correlations were found

Table 3

Correlation analysis between different nutritional components and antioxidant activities

Index	SPC	TAA	TSC	TPC	TFC	DPPH	ABTS	FRAP	FICA
SPC	1								
TAA	0.478	1							
TSC	-0.681**	-0.839**	1						
TPC	0.552*	0.653**	-0.827**	1					
TFC	0.113	0.859**	-0.611**	0.406	1				
DPPH	0.007	0.832**	-0.493	0.314	0.958**	1			
ABTS	0.697**	0.868**	-0.902**	0.752**	0.744**	0.625*	1		
FRAP	0.222	0.175	-0.226	0.373	-0.029	-0.004	0.248	1	
FICA	0.707**	0.853**	-0.860**	0.800**	0.547*	0.530*	0.845**	0.407	1

SPC – soluble protein content, TAA – total amino acids, TSC – total starch content, TPC – total phenolic content, TFC – total flavonoid content, FRAP – ferric reducing antioxidant power, FICA – ferrous ion chelating activity, * significantly correlated at the 0.05 level (bilateral), ** significantly correlated at the 0.01 level (bilateral)

among the various methods used to determine the antioxidant potential, especially between the ABTS and FICA assays (R^2=0.845, p<0.01). The results showed that total amino acids and the soluble protein content had positively significant correlation with ABTS and FICA activities both in seeds and in sprouts (Table 3). Conversely, the total starch content showed negative correlation with total phenolics, total flavonoids and antioxidant activity. It is interesting that the total phenolic and total flavonoid content showed a similar positively significant correlation with ABTS and FICA activities, especially the high content of phenolic compounds was strongly positively correlated to their DPPH radical scavenging activity (R^2=0.958, p<0.01). Meanwhile, the FRAP showed weaker correlations with nutrient compounds, and the three antioxidant activities.

DISSCUSION

During the germination process, an array of morphological, biochemical, and physiological changes is strongly correlated with the survival and growth rate of seedlings, which ultimately affects quality and yield (Zaynab et al. 2021). Many studies have shown that germination significantly affects the color, endosperm and cotyledon characteristics of sprouts, and the color and hypocotyl of cotyledon increased appreciably with more prolonged germination period (Zhang et al. 2015, Singh et al. 2019). Similar results were achieved in our experiment (Figure 1). The fundamental role of water in plant life is well known. Under suitable conditions, dry seeds gradually resume metabolic activities by absorbing water from the environment, enabling them to complete essential cellular events and to prepare for subsequent seedling growth (Liu et al. 2022). Therefore, water is helpful in the maintenance of the protoplasmic content of cell and leaf texture, and is necessary for the metabolic activity of plants. Our results show that the moisture content of germinated seeds rapidly increased to 78.8% at 8 d of germination. A previous study showed that the moisture content of buckwheat sprouts increased above 57.2% with the increase of germinating time (Zhang et al. 2015). Our results are consistent with the cited study, demonstrating that the high moisture content in *F. platanifolia* sprouts enables them to carry out active metabolism (Figure 2*a*).

Protein, the material basis of all life, plays a critical role in the life activities of cells and organisms. During germination, storage proteins are hydrolyzed under the action of proteases to release amino acids or small molecular peptides (Liu et al. 2022). The present study revealed that germination could significantly increase the soluble protein content of *F. platanifolia* (Figure 2*b*). Our findings are in line with previous research on the soluble protein content of buckwheat, which significantly increased as the germination time increased (Zhang et al. 2015). This result seems to indicate that protein synthesis outpaced the effect of proteolysis of *F. platanifolia* seeds during germination, and the decrease of carbohydrates may also account for this increase in the protein content. However, previous research suggested a decrease in the protein content in germinated legumes (kidney, mung, soy bean, and peanut) due to protein catabolism (Megat et al. 2011). Hence, the protein content in germinated seeds depends on the balance between protein degradation and protein biosynthesis during germination, which varies among seed species. Besides, the total amino acid content is an essential parameter in investigating the qualities of foods and crops as well as their nutritive value (Zhao et al. 2021). The current study illustrated that the total amino acid content in germinated *F. platanifolia* increased with an increase in germination time (Figure 2*c*). Similar results have been observed by Ha et al. (2017) and Qi et al. (2022), which might be due to the hydrolysis of proteins to release amino acids or small molecular peptides.

Starch is the main form of nutrient storage in edible seeds and the main reservoir of energy for biosynthesis (Singh et al. 2019). Legume seeds cannot obtain energy from the outside world during germination, so they must degrade their own storage substances to provide energy for growth (Liu et al. 2022). Our research has demonstrated that the extent of starch hydrolysis and reducing sugar content of germinated *F. platanifolia* also increased gradually throughout the period of germination (Figure 3*a*). These results were similar to the ones reported by Liu et al. (2022). This was due to the hydrolysis of starch by the enzyme amylase along with a combined action of invertase, which assists in the complete hydrolysis of starch to reducing sugar, yielding sucrose and fructose.

Minerals are dietary requirements for humans, and they exert various physiological effects, which are very important for maintaining an overall physical and mental well-being, and are necessary for the normal functioning of an organisms (Angeli et al. 2020). Many studies have indicated that the various mineral concentrations in sprouted seeds (moringa and lentil) showed a different degree of decrease as compared with non-germinated seeds during germinating process (Coello et al. 2020, Alkaltham et al. 2022). Similar results were observed in our experiment (Table 2). In our study, the lower content of minerals in germinated seeds might be due to the leaching out of some minerals into the soaking water, as well as to increasing phytate enzyme activity, resulting in consumption of minerals during germination. At the same time, it is noteworthy that different varieties of minerals have different changing trends during germination, suggesting that the seeds and sprouts of *F. platanifolia* are selective in absorption and utilization of mineral elements.

Total phenolics are naturally produced during the growth and development of plants, and they are the main secondary metabolites in most plants, which provide essential functions in the germination and growth of the plants (Pająk et al. 2014). Our study suggests that the germination process may cause significant changes in the phenolic content, and the germination significantly increased total phenolic compounds in *F. platanifolia* seeds in a time-dependent manner (Figure 4*a*). This research supports the theory that the phenolic content positively increases with germination, as shown by legumes and cereals. The significant increase of the total phenolic content could be because that the seed's respiratory system is active during germination, and different proteolytic, amylolytic, and cell wall degradation enzymes that contributed to the release of bound phenolic compounds could be produced (Acosta-Estrada et al. 2014). Another reason could be because the enzyme phenylalanine ammonia lyase (PAL) is highly active and could promote the biosynthesis of phenolic compounds during germination (Duodu 2014). Conversely, in some cases, germination causes a significant reduction of phenolic compounds. Phenolics are usually lost during the soaking of seeds, but may also be used by growing plants as precursors of cell wall

components, hormones, and other regulatory compounds (Pająk et al. 2019). Qi et al. (2022) reported an increasing trend of flavonoids in soybean seeds during germination, which is consistent with our findings (Figure 4*b*). Similar increases in the total flavonoid content during germination period were also reported by Pająk et al. (2014) and Kim et al. (2012) for mung beans. This increase could be attributed to the synthesis of flavonoid compounds during germination.

The excessive ROS causes damage to DNA, RNA and cell organelles, but antioxidant components help to scavenge the free radicals generated in the body at the right time and right place through the neutralization of free radicals and reactive oxygen species in the cell. Thus, antioxidant components play a very important role in the health of humans (Anju et al. 2022). DPPH is a very stable free radical and acts as a representative reactant in measuring the antioxidant capacity. ABTS has been identified as a flexible method to estimate free radical scavenging activity and can determine antioxidant capacity of both hydrophilic and lipophilic compounds. FRAP is evaluated by the Fe^{3+} to Fe^{2+} transformations and acts as an important indicator of antioxidant activity. In the present study, we have found that the change trend of each antioxidant activities in different germinated seeds differ slight (Figure 5). This may due to the different antioxidant reaction mechanisms. Meanwhile, it was found that the DPPH and ABTS of seeds showed similar variation, while the ABTS had much greater free radical scavenging activity than DPPH during germination, suggesting that ABTS radicle cations were the mayor contributors to the total antioxidant activity. Moreover, it was shown that germination promotes the antioxidant potential in plant seeds, and significant increments of antioxidant activities were observed during germination (Zhang et al. 2015). This was probably due to the release of bound phenolic compounds from the breakdown of cellular constituents and cell walls. However, Shohag et al. (2012) reported that hydrophilic antioxidant capacity in soybean and mung bean seeds decreased with germination time. The result may be due to the decrease in the phenolic substance content due to the activation of hydrolase and polyphenol oxidase activities during germination. The difference may be related to the time of germination.

The Pearson's correlation analysis was performed between the nutrient compounds and antioxidant activity to determine the major contributors to the antioxidant activity during germination time. The high content of phenolic compounds of *F. platanifolia* seeds was strongly related to their ABTS radical scavenging activity as well as to the ferrous ion chelating activity (Table 3). The antioxidant activity was also positively correlated with the content of total flavonoids, which indicates that all these compounds contributed to the antioxidant capacity of *F. platanifolia* seeds (Table 3). Gomez-Favela et al. (2017) observed a similar increasing trend of phenolic compounds and antioxidant activity (ABTS method), and also found a high

correlation between total phenolics and antioxidant activity. Our results have confirmed this observation, indicating that phenolic compounds are mainly responsible for the antioxidant activity of seeds and sprouts.

CONCLUSION

A clear pattern of changes in the morphological characteristics, nutrient compounds, and antioxidant activity of *F. platanifolia* seeds at different germinating times is presented in this study. The results demonstrated that the content of water, soluble protein, total amino acid, reducing sugar, TPC, TFC and DPPH in *F. platanifolia* increased significantly with the increasing germination time, while the content of total starch and mineral element decreased significantly. Germination caused significant changes in the biochemical and nutritional compositions of *F. platanifolia* seeds. The significantly higher content of nutritional components and antioxidant activity was observed in germinated *F. platanifolia* seed than their non-germinated forms. Therefore, germinated *F. platanifolia* seeds are an excellent source of nutritional substances, especially phenolic and flavonoids compounds, and they have great antioxidant activity.

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