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Microwave Irradiation Enhances the Germination Rate of Tartary Buckwheat and Content of Some Compounds in Its Sprouts

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Key words: tartary buckwheat, microwave treatment, germination, sprouts

Tartary buckwheat (*Fagopyrum tataricum*) seeds were irradiated with microwaves at various power levels of 200, 400, 600 and 800 W for 10 or 30 s. The irradiated grains were germinated for 3, 5, and 7 days and harvested. The germination rate of the tartary buckwheat seeds and contents of some compounds in the sprouts were investigated. The results showed that the exposure to 600 W microwaves for 10 s resulted in the highest final germination rate after 7 days of germination, which was 2 times that of the control. The exposure of seeds to 800 W for 30 s showed the lowest germination rate (approximately 10%), which decreased by 87% compared with the control ($p<0.05$). The exposure at 600 W for 30 s stimulated the total flavones content, reduced the sugar and soluble protein contents, and increased the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The highest free amino acid content (11 mg/g) was observed in 5-day sprouts exposed to 800 W for 10 s. Moreover, the microwave treatment had a positive effect on the catalase (CAT) and superoxide dismutase (SOD) activity.

INTRODUCTION

Seed germination is the most important period in the life cycle of a higher plant and involves a series of morphological, physiological and biochemical changes. The germinated grain has improved digestibility and bio-availability of nutrients; for example, after germination vitamin content increases [Rajjou *et al.*, 2012], and the amino acid composition is regulated to change more reasonably. Moreover, the contents of γ-aminobutyric acid (GABA) [Guo *et al.*, 2016], free amino acids, phenolics, and other active ingredients that have a unique nutritional value and health functions in the sprouted grain are significantly diversified [Doblado *et al.*, 2007].

Tartary buckwheat (*Fagopyrum tataricum*) is a medicinal and edible plant that is extremely rich in bioactive ingredients such as flavonoids [Li *et al.*, 2016; Fabjan *et al.*, 2003] and GABA. The flavonoid rutin increases after germination so that the nutritional value and the biological activity are significantly improved [Zhang *et al.*, 2015; Nam *et al.*, 2015]. Most non-invasive techniques include treatment with electromagnetic waves, particularly microwaves, optical emissions, magnetic fields, ultrasound and blue light – UV radiation, which has been used in agriculture to improve seed germination and increase the yield of biologically-active components. Some previous studies have indicated that physical treatments, including magnetic fields [Zhou *et al.*, 2015a], electric fields, supersonic [Onac *et al.*, 2016; Yu *et al.*, 2016], and high pressure treatments [Doblado *et al.*, 2007], induce effects in the plant tissue [Carbonell *et al.*, 2000], thereby regulating the progress of germination [Kadlec *et al.*, 2001; Aladjadjiyan, 2012]. These physical factors can stimulate phenylalanine ammonialyase (PAL) and chalconeisomerase (CHI) during the germination of buckwheat seeds to increase flavonoid synthesis [Roux *et al.*, 2006; Pelletier *et al.*, 1999]. The activities of the two key enzymes, PAL and CHI, have a close relationship with the total flavonoid content during the germination of buckwheat sprouts [Li *et al.*, 2011]. The two enzymes act synergistically during the time course of both dark and light conditions to yield the maximum total flavonoid content in tartary buckwheat sprouts [Thwe *et al.*, 2014; Ji *et al.*, 2016]. The activity of PAL and CHI was also in accordance with the variety of total flavonoid content. Therefore, light [Ji *et al.*, 2016] and magnetic field [Asghar *et al.*, 2016] can influence flavonoid content by changing the activity of PAL and CHI. Microwaves are a form of electromagnetic radiation, with a frequency between 30 and 300 GHz, that has been shown to significantly stimulate germination [Gaurilcikiene *et al.*, 2013]. As a type of electromagnetic wave, a microwave at a certain power can effectively activate various enzymes involved in seed germination [Radzevičius *et al.*, 2013], significantly improving the germination rate (GR) and increasing the synthesis of certain biological components in the seeds [Stan *et al.*, 2014]. Microwave pretreatment promotes the expression of the genes encoding peroxidase (POD) and superoxide dismutase (SOD) isozymes in plant seedlings [Aladjadjiyan, 2012] to significantly increase the germination potential, GR, stem length, root length and the total mass

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of the seeds [Radzevičius *et al.*, 2013]. Moreover, this increase was positively correlated with the microwave power [Łupinska *et al.*, 2009; Han, 2010]. The protein and amino acid contents of wheat seedlings increased when the seeds were pretreated with microwaves, and the increase was microwave irradiation dose-dependent [Hamada, 2007]. The dose- dependency may be related to the changes in the protein structure of the enzyme due to the microwave treatment [Damm *et al.*, 2012].

A previous study showed that microwave pretreatment of broad beans increased the phenolic content and levodihydroxy phenylalanine (l – DOPA) in seedlings by 700% and 59%, respectively [Randhir & Shetty, 2004]. Microwave pretreatment of rice and millet increased the tocopherol content of the germ oil of rice by 150% and the phytosterol content by 15%, and it slightly increased the GABA content [Kwon *et al.*, 2004]. Moreover, the acceptable biological polyphenol content of the millets increased significantly [Hithamani & Srinivasan, 2014]. Wheat (*Triticum aestivum* L. Sakha 61) seeds irradiated with microwaves for a long time (75 min) showed a 7-fold higher carotenoid content in the seedlings than in the control sample 7 days after germination. The protein and amino acid contents also increased, and the total sugar content decreased. However, treatment for a shorter time (15 min) stimulated proline synthesis in the seedlings [Hamada, 2007]. Microwave irradiation of brown rice significantly increased GABA, the total phenolic content and the DPPH RSA (radical scavenging activity, RSA) [Seo *et al.*, 2016], indicating that microwaves can increase the secondary metabolite content and the nutritive value of sprouts and grains and thereby improve the quality of the germinated grain [Uppal & Bains, 2012].

Pretreatment with microwaves activates the POD and SOD isozymes at some gene loci and enhances their expression in seedlings [Aladjadjiyan, 2012]; therefore, they can maintain the integrity of the cell membrane and the antioxidant capacity, which enhances plant stress resistance [Chen *et al.*, 2009a]. Seeds irradiated with microwaves followed by salt stress restored plant growth parameters and biochemical parameters related to an oxidative status almost to the levels of non-salt-treated seedlings. The results indicated that irradiating wheat seeds with a suitable dose of microwave radiation could enhance the tolerance of seedlings to salt stress [Chen *et al.*, 2009a; Chen *et al.*, 2009b]. Microwaves improve the activities of some enzymes in *Isatis indigotica* cotyledons, including amylase, alanine aminotransferase and protease, and they accelerate cell metabolism and significantly increase biophoton emissions [Chen *et al.*, 2005]. Ultrasound stress [Dhawi & Al Khayri, 2011] induced the expression of the PAL and CHI genes. To adapt to the invasive stress conferred by microwaves, plants regulate the activity of certain enzymes and improve the content of osmolytes, flavonoids and proline.

The studies mentioned above showed that pretreatment with microwaves promotes seed germination and early metabolism; however, there are a few studies on seeds regarding short-term (effects on seed germination) or long-term effects (effects on seedling growth, metabolism and biomass accumulation) of the dosage and treatment times of microwaves, particularly in buckwheat seeds. Therefore, the purpose of this study was to investigate the effects of microwave irradiation on the germination characteristics of tartary buckwheat seeds and the physiological and biochemical indexes in the germinating seeds.

MATERIAL AND METHODS

Materials and chemical reagents

Ning Qiao NO.2 tartary buckwheat seeds (*Fagopyrum esculentum*) were obtained from the Yanchi seed company in NingXia province of China. Chemical reagents such as rutin (purity >99%) and the other reagents were purchased from the Sinopharm Chemical Reagent Co., Ltd. The microwave source was a Japanese Panasonic NN – K597WS microwave oven (modified for this study) with variable power from 200 to 900 W and a frequency of 2450 MHz.

Germination procedures

Tartary buckwheat seeds were sterilized with a 1.0 g/L potassium permanganate solution for approximately 5 min at room temperature (30°C) and washed three times with distilled water. Before germination, the seeds were soaked in distilled water at 30°C for 4 h, and the water was changed once again during this period. The seeds were transferred to warm water (55±1°C) for approximately 15 min to induce germination.

Microwave pretreatment methods

Tartary buckwheat seeds were artificially germinated; then, 50 grams of seeds were separated into three groups and placed in 9-cm diameter culture dishes. Each group contained approximately 50 ears (approximately 1.0 gram). The seeds were placed in a microwave oven for irradiation by microwaves. The tartary buckwheat grains were exposed to microwave irradiation at 200, 400, 600 and 800 W for 10 or 30 s, and they were covered with two pieces of filter paper. The experiment consisted of nine treatments of microwave irradiation: 0 s (CK, the control), 200 W for 10 s and 30 s, 400 W for 10 s and 30 s, 600 W for 10 s and 30 s, and 800 W for 10 s and 30 s. The seeds were incubated at 85% RH and 25±2°C for 7 days in a seed germination incubator. The seeds were rinsed with 8 mL distilled water every 12 h during the incubation period. Seeds not treated with microwave were used as the control (CK). The sprouts were promptly removed after germination and stored at – 80°C until use.

Germination rate

From the beginning of incubation, the GR of the seeds was measured every 12 h (germination was defined as a sprout length of greater than 1/2 the seed length). The average was calculated (GR = germinating seeds/total seeds×100%), and the GR was evaluated for the subsequent 7 days. The final GR was the germination after 7 days.

Total flavonoids

Content of total flavonoids was determined by a sodium nitrite-aluminum nitrate colorimetric method based on the method of Ji *et al*. [2016] with some modifications. One gram of freeze-dried tartary buckwheat sprouts was placed into a mortar and immersed in 5 mL 60% (v/v) ethanol; some

quartz sand was added, and the mixture was evenly ground. An additional volume of 60% (v/v) ethanol was added to bring the solution to 50 mL, which was centrifuged at 9000×g for 10 min. The supernatant was the sample extract. Next, 1 mL of the sample extract was transferred to a 10-mL volumetric flask, and 2 mL of 0.1 M aluminum chloride solution and 3 mL of 1 M potassium acetate were added. The mixture was diluted to 10 mL with 60% (v/v) ethanol, mixed well and maintained in the dark for 30 min. The absorbance was measured at 420 nm. Rutin was used to create a standard curve, and the total flavonoids were expressed as rutin equivalents [Ji *et al.*, 2016].

$$A_{420} = 4.0556 \text{ (mg/mL) rutin} + 0.0003, R^2 = 0.999 \quad (1)$$

Contents of dry matter, total soluble sugar, reducing sugar, soluble protein, and free amino acids

Contents of dry matter, total soluble sugar, reducing sugar, soluble protein and free amino acids in tartary buckwheat seeds during sprouting was determined according to the following methods. Dry matter content (%) was determined after drying at 60°C to a constant weight, with a reproducibility rate of 0.02%. The total soluble sugar and soluble protein contents were estimated as described by Wang *et al*. [2001]. The free amino acid content was determined according to the method of Aragão *et al*. [2015], whereas the content of reducing sugars was measured according to the standard AOAC methods 939.03 [AOAC, 1995].

Catalase and superoxide dismutase activity

The enzymes were extracted from 0.5 grams of seedlings using a mortar and pestle with 5 mL extraction buffer containing 50 mM potassium phosphate buffer (pH 7.6) and 0.1 mM disodium ethylenediamine tetraacetate (Na_2-EDTA). The homogenate was centrifuged at 15,000×*g* for 15 min, and the supernatant was assayed for the various enzymes. All steps in the preparation of the enzyme extracts were performed at 4°C in an ice bath. SOD was assayed according to Fikret *et al.* [2013] by monitoring the superoxide radical-induced reduction of nitro blue tetrazolium (NBT) at 560 nm. One unit of SOD activity was defined as the amount of enzyme that caused a 50% inhibition in the photochemical reduction of NBT. The catalase (CAT) activity was determined by monitoring the disappearance of H_2O_2 according to the method of Fikret *et al.* [2013].

DPPH radical scavenging activity

The DPPH radical scavenging activity (RSA) was measured according to the method described by Zhou *et al*. [2015b] and Ji *et al*. [2016] with some modifications. Briefly, 0.2 mL of a previously prepared 60% (v/v) ethanol extract was added to 7.8 mL, 0.025 mg/mL DPPH in ethanol. The mixture was incubated at room temperature in the dark for 30 min, and the absorbance was measured at 517 nm (A_T). A pure DPPH solution was used as the control, and its absorbance at 517 nm was set as A_0. The DPPH RSA was calculated as follows [Ji *et al.*, 2016]:

$$\text{RSA (\%)} = (A_0 - A_T) \times 100/A_0 \quad (2)$$

Statistical analysis

Quantitative analyses were performed in triplicate on sprouts treated and untreated with microwaves. The data were expressed as the mean and standard deviation and analyzed with one-way ANOVA followed by Duncan's multiple range test. The significance level was $P<0.05$. The software program SPSS (SPSS Inc., Chicago, IL, USA) was used for the analyses.

RESULTS

Effect of microwaves on the germination rate

Figure 1 shows the GR of tartary buckwheat seeds after irradiation at different power levels for different times. Microwaves (200 to 800 W) applied for 10 s or 30 s significantly ($P<0.05$) affected the germination of tartary buckwheat seeds. The GR after 2 days was 47% when the seeds were exposed to 600 W microwaves for 10 s, which showed a 54%

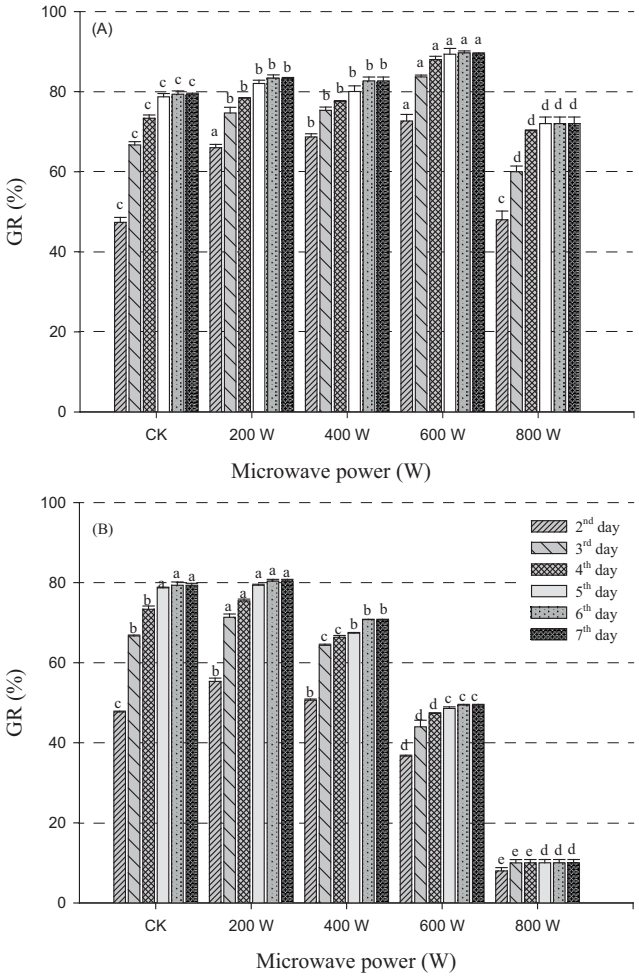
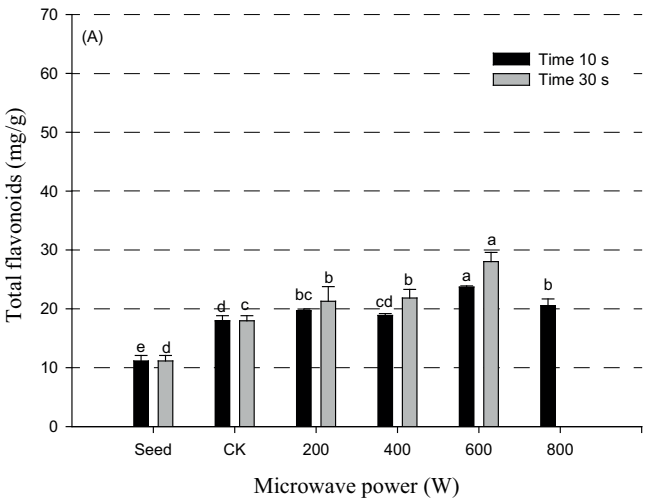
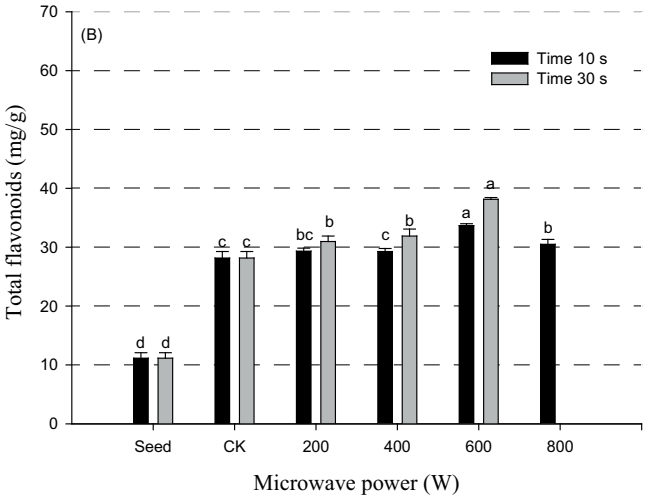


FIGURE 1. Changes in the germination rate of tartary buckwheat sprouts in response to different microwave treatments.

(A) Treatment time 10 s; (B) Treatment time 30 s. All values are the means±SD (n=50). Values of the GR among microwave treatments with different power levels shown with different lowercase letters are significantly different by ANOVA with Duncan's multiple range test at $P<0.05$. CK is the control. Seeds that were treated with microwaves at 800 W for 30 s had the lowest GR. The data of the test for the contents and enzyme activity is not shown.





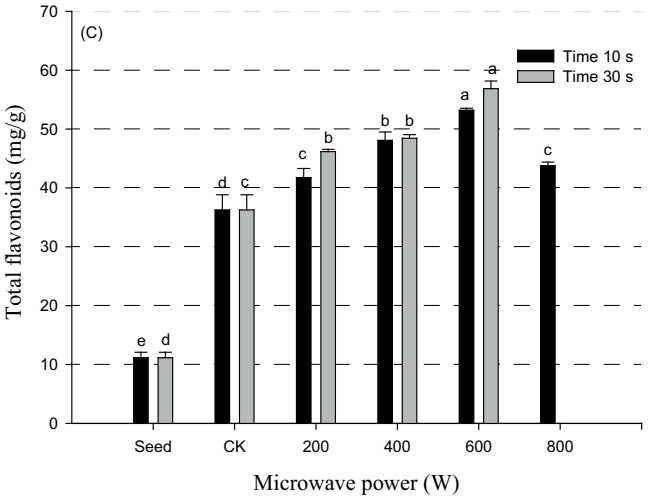


FIGURE 2. Changes in the total flavonoid content of tartary buckwheat sprouts in response to different microwave treatments.

(A) 3-day sprout; (B) 5-day sprout; (C) 7-day sprout. All values are the means±SD (n=50). Values of the total flavonoid content with different microwave power levels shown with different lowercase letters are significantly different by ANOVA with Duncan's multiple range test at $P<0.05$. CK is the control. Seeds that were treated with microwaves at 800 W for 30 s had the lowest GR. The data of the test for the total flavonoid content is not shown.

increase compared with the control. The GR at 800 W microwave exposure for 10 s was 48%, but no significant difference ($P>0.05$) was observed compared with the control. After incubation for 7 days, the GR of seeds exposed to 600 W for 10 s was the highest (89%), which increased 13% compared with the control. The GR of seeds exposed to 800 W for 10 s was the lowest (72%), which was a significant decrease (10%) compared with the control ($P<0.05$).

The results in Figure 1 show that the GR decreased with an increase in the microwave power from 200 W to 800 W for 30 s when the seeds were incubated for 2 days. When the microwave power was 200 W for 30 s, the GR was 55%, showing an increase of 17% ($P<0.05$) compared with the control. At 800 W for 30 s, the GR was only 8%, which indicates a significant reduction of 83% compared with the control ($P<0.05$). The highest GR (80%) was observed after 7 days at 200 W for 30 s.

Effect of microwaves on total flavonoids

As shown in Figure 2, three days after the microwave treatment at 600 W for 10 s, the total flavonoid content of the sprouts was 24 mg/g, which showed 113% and 32% increases compared with seeds and the control, respectively ($P<0.05$). The total flavonoid content after the microwave treatment of 600 W for 30 s was 28 mg/g, which showed an increase of 151% and 56% compared with the seeds and the control, respectively. The total flavonoid content of sprouts after the microwave treatment of 30 s was slightly higher than after 10 s and the same microwave power (Figure 2 A). After 5 days (Figure 2 B), the total flavonoid content of sprouts treated with 600 W for 10 s was 34 mg/g, showing an increase of 202 % and 20 % compared with seeds and the control, respectively. At 600 W for 30 s, the total flavonoid content was 38 mg/g, indicating an increase of 242% and 35% compared with the seeds and control ($P<0.05$). Finally, a treatment of 600 W for 10 s produced 53 mg/g of total flavonoids in the sprouts after 7 days, which indicated an increase of 377% and 89% compared with that in seeds and the control (Figure 2 C). However, at 600 W for 30 s, the sprouts showed the highest total flavonoid content of 57 mg/g ($P<0.05$).

Effect of microwave treatment on contents of total soluble sugars and reducing sugars

As shown in Table 1, the total soluble sugar content of sprouts decreased with an increase in microwave power ($P<0.05$). After 7 days, sprouts treated with microwave at 800 W for 10 s had a total soluble sugar content of 134 mg/g. Compared with seeds and the control, the total soluble sugar content decreased by approximately 23% and 9%, respectively. For the microwave treatment of 600 W for 30 s, the total soluble sugar content was 132 mg/g, showing a decrease of 24% and 11% compared with seeds and the control. However, for a power of 600 W for 30 s, the total soluble sugar content in the sprouts decreased to 132 mg/g ($P<0.05$).

Microwave treatment resulted in significant differences ($P<0.05$) in the content of reducing sugars in sprouts (Table 1). An increase in the microwave power from 200 to 800 W first increased then decreased the reducing sugar content of the sprouts. For a microwave treatment of 600 W ap-

TABLE 1. Changes in the total soluble sugar and reducing sugar contents of tartary buckwheat sprouts with different microwave treatments.

Microwave power/W	The soluble total sugar content (mg/g)						The reducing sugar content (mg/100 g)					
	3rd day		5th day		7th day		3rd day		5th day		7th day	
	10 s	30 s	10 s	30 s	10 s	30 s	10 s	30 s	10 s	30 s	10 s	30 s
Seed	175.30 ± 2.72^{aA}	175.30 ± 2.72^{aA}	175.30 ± 2.72^{aA}	175.30 ± 2.72^{aA}	175.30 ± 2.72^{aA}	175.30 ± 2.72^{aA}	0.35 ± 0.01^{fA}	0.35 ± 0.01^{eA}	0.35 ± 0.01^{fA}	0.35 ± 0.01^{dA}	0.35 ± 0.01^{eA}	0.35 ± 0.01^{dA}
CK	160.35 ± 0.07^{bA}	160.35 ± 0.07^{cA}	154.28 ± 0.74^{cA}	154.28 ± 0.74^{cA}	149.31 ± 1.74^{cA}	149.31 ± 1.74^{bA}	6.64 ± 0.46^{eA}	6.64 ± 0.46^{dA}	11.31 ± 0.19^{eA}	11.31 ± 0.19^{cA}	20.60 ± 0.55^{dA}	20.60 ± 0.55^{cA}
200	164.10 ± 1.70^{bA}	161.67 ± 1.61^{bA}	157.77 ± 2.56^{bA}	154.10 ± 1.09^{bA}	153.54 ± 2.45^{bA}	150.17 ± 1.97^{bA}	9.46 ± 0.47^{dB}	10.66 ± 0.47^{cA}	12.79 ± 0.54^{dB}	17.58 ± 0.24^{bA}	21.65 ± 0.23^{cB}	23.45 ± 0.61^{bA}
400	160.78 ± 2.05^{bA}	155.24 ± 1.89^{cdB}	154.44 ± 1.25^{cA}	151.42 ± 1.65^{bcA}	144.58 ± 1.38^{dA}	140.92 ± 1.41^{cB}	11.42 ± 0.58^{cA}	12.14 ± 0.35^{bA}	14.09 ± 0.02^{cB}	18.28 ± 0.18^{aA}	22.84 ± 0.78^{bA}	20.53 ± 0.06^{cB}
600	154.07 ± 3.24^{cA}	153.45 ± 1.02^{dA}	147.97 ± 0.69^{dA}	144.47 ± 0.66^{dB}	139.65 ± 1.71^{eA}	132.42 ± 1.07^{dB}	14.42 ± 0.28^{aB}	15.52 ± 0.58^{aA}	16.23 ± 0.05^{aB}	17.44 ± 0.59^{bA}	24.71 ± 0.40^{aA}	25.93 ± 0.80^{aA}
800	150.55 ± 0.25^{c}	–	144.18 ± 1.48^{e}	–	134.47 ± 1.60^{f}	–	13.15 ± 0.59^{b}	–	14.82 ± 0.11^{b}	–	20.54 ± 0.08^{d}	–

All values are the means±SD. Values within a row with different lowercase letters are significantly different by ANOVA with Duncan's multiple range test at $P<0.05$. Values within a line (10 s and 30 s) with different uppercase letters are significantly different by ANOVA with Duncan's multiple range test at $P<0.05$. CK is the control.

TABLE 2. Changes in the soluble protein and free amino acid content of tartary buckwheat sprouts in response to different microwave treatments.

Microwave power/W	The soluble protein content (mg/100 g)						The free amino acid content (mg/g)					
	3rd day		5th day		7th day		3rd day		5th day		7th day	
	10 s	30 s	10 s	30 s	10 s	30 s	10 s	30 s	10 s	30 s	10 s	30 s
Seed	25.27 ± 0.14^{dA}	25.27 ± 0.14^{eA}	25.27 ± 0.14^{eA}	25.27 ± 0.14^{dA}	25.27 ± 0.14^{fA}	25.27 ± 0.14^{dA}	2.13 ± 0.22^{eA}	2.13 ± 0.22^{eA}	2.13 ± 0.22^{eA}	2.13 ± 0.22^{dA}	2.13 ± 0.22^{eA}	2.13 ± 0.22^{dA}
CK	85.70 ± 1.56^{cA}	187.64 ± 1.44^{dA}	111.46 ± 0.18^{bA}	111.53 ± 0.30^{cA}	108.92 ± 1.12^{cA}	108.78 ± 1.24^{cA}	4.17 ± 0.18^{dA}	4.17 ± 0.18^{dA}	6.51 ± 0.20^{dA}	6.51 ± 0.20^{cA}	5.43 ± 0.26^{dA}	5.43 ± 0.26^{cA}
200	85.12 ± 0.10^{cB}	90.66 ± 0.47^{cA}	103.22 ± 0.39^{dB}	118.25 ± 1.38^{bA}	101.07 ± 0.83^{eB}	114.58 ± 3.23^{bA}	5.30 ± 0.20^{cB}	5.80 ± 0.15^{cA}	7.55 ± 0.23^{cB}	8.22 ± 0.35^{bA}	6.31 ± 0.11^{cA}	6.64 ± 0.40^{bA}
400	97.42 ± 4.50^{bA}	101.81 ± 1.43^{bdA}	108.76 ± 3.21^{cB}	118.95 ± 1.08^{bcA}	113.90 ± 0.45^{dB}	116.28 ± 0.86^{abA}	5.55 ± 0.26^{cB}	7.19 ± 0.26^{bA}	7.71 ± 0.09^{cB}	8.74 ± 0.09^{bA}	6.78 ± 0.14^{cA}	7.18 ± 0.22^{bA}
600	111.42 ± 1.26^{aA}	114.52 ± 2.31^{aA}	116.23 ± 0.05^{aB}	121.77 ± 2.08^{aA}	111.66 ± 0.47^{aB}	119.06 ± 2.09^{aA}	6.51 ± 0.37^{bB}	8.54 ± 0.41^{aA}	9.28 ± 0.45^{bB}	10.33 ± 0.33^{aA}	8.11 ± 0.50^{bA}	8.68 ± 0.24^{aA}
800	111.15 ± 1.60^{a}	–	114.82 ± 0.11^{a}	–	134.47 ± 1.60^{b}	–	8.40 ± 0.35^{a}	–	11.24 ± 0.38^{a}	–	10.09 ± 0.26^{a}	–

All values are the means±SD. Values within a row with different lowercase letters are significantly different by ANOVA with Duncan's multiple range test at $P<0.05$. Values within a line (10 s and 30 s) with different uppercase letters are significantly different by ANOVA with Duncan's multiple range test at $P<0.05$. CK is the control.

plied for 10 s, the reducing sugar content of 3-day sprouts was 14.42 mg/100 mg, showing a 40-fold and 117% increase compared with that of seeds and the control, respectively. For a microwave treatment of 600 W for 30 s after 5 days, the reducing sugar content was 18 mg/100 mg, showing an increase of approximately 13% compared to exposure to 600 W for 10 s (Table 1). After 7 days (Table 1), a microwave treatment at 600 W for 30 s produced the highest reducing sugar content of 26 mg/100 mg in the sprouts, which increased 72-fold and 26% compared with that in seeds and the control, respectively ($P<0.05$).

Effect of microwave treatment on contents of soluble protein and free amino acids

As shown in Table 2, the soluble protein content in the sprouts first increased; then it decreased when the microwave power was increased from 200 to 800 W. The soluble protein content of 3-day sprouts irradiated with 600 W for 10 s was 111 mg/100 mg, which showed 341% and 27% increases compared with that of seeds and control ($P<0.05$). The 600 W for 30 s treatment increased soluble protein content approximately 3% more than that of 600 W for 10 s in the sprouts that were cultured for 5 days. The soluble protein content of sprouts irradiated with 600 W for 30 s increased approximately 9% compared with that of 600 W for 10 s and reached 122 mg/100 mg. However, the soluble protein content of 7-day tartary buckwheat sprouts reached 119 mg/100 mg at 600 W for 30 s. However, it increased by only approximately 370% and 9% compared with that of seeds and the control ($P<0.05$).

The free amino acid content is shown in Table 2. It increased in sprouts with an increase in microwave power from 200 to 800 W. For a microwave treatment of 800 W for 10 s, the 5-day sprouts had the highest free amino acid content (11 mg/g), showing an increase of quadruple and 72% compared with that of seeds and the control. At microwave powers of 200, 400 or 600 W, the free amino acid content of sprouts treated for 30 s compared with 10 s increased by 9%, 13% and 11%, respectively. After 7 days, the free amino acid content of the sprouts was less than that after 5 days ($P<0.05$).

Effect of microwave treatment on the catalase and superoxide dismutase activity

As shown in Figure 3 (A, B and C), the effect of different microwave treatments on the CAT activity was significant ($P<0.5$). When seeds were treated with microwaves at 600 W for 30 s, the CAT activity in the seedlings peaked at 18.00, 27.53 and 30.70 mg H_2O_2/g FW × min after 3, 5 and 7 days, respectively. The CAT activity of the seedlings showed 4-, 4- and 3-fold increases compared to the control, respectively. The CAT activity increased with an increase in microwave power. At the same power, the CAT activity increased significantly when the treatment time was changed from 10 s to 30 s ($P<0.5$).

Microwave treatment had a significant effect on SOD activity ($P<0.5$), as shown in Figure 3 (D, E and F). After germination for 3, 5 and 7 days at 600 W for 30 s, the SOD activity peaked approximately at 18, 22 and 23 U/g, respectively. It increased by 58%, 34% and 39%, respectively, compared with that of the control. The SOD activity increased with an increase in microwave power. At the same power, the SOD activity significantly increased when the treatment was extended from 10 s to 30 s ($P<0.5$). The SOD activity significantly increased from the 3rd to the 5th day, and this increase was reduced after 5 days.

Effect of microwave treatment on DPPH radical scavenging activity

As shown in Figure 4, when the microwave power was increased from 200 to 800 W, the DPPH RSA also increased. For 7-day sprouts, the DPPH RSA for the 800 W for 10 s exposure was 62%, showing an increase of 264% compared with seeds, while an exposure of 600 W for 30 s produced an activity of 67%. Compared with a 10-s treatment time, the DPPH RSA after 30 s increased by 4%, 5% and 7% at 200, 400, and 600 W, respectively ($P<0.05$).

DISCUSSION

Plant growth and development is modulated by internal cues and external environmental factors because plants are particularly sensitive to external environmental factors. The effects of microwave irradiation are based on the effects of temperature and electromagnetism on organisms. Previous studies have illustrated that certain doses of microwave irradiation notably improve the activities of nitric oxide synthase (NOS), CAT, POD, and SOD and increase the concentration of nitric oxide (NO), ascorbic acid (VC) and glutathione (GSH) [Chen *et al.*, 2009a]. Radzevicius *et al*. [2013] reported that radish and carrot seeds exposed to 9 GHz microwaves (80 kW) had 26% and 11% higher GR, respectively. According to our results (Figure 1), the lowest GR of 10% was observed at 800 W for 30 s, which was 87% lower compared with the control ($P<0.05$). The GR of tartary buckwheat seeds significantly increased ($P<0.05$) as the microwave power increased but was significantly reduced when the exposure time was increased from 10 s to 30 s at higher irradiation power (>600 W) (Figure 1). These results were in accordance with previous studies in wheat [Ragha *et al.*, 2011; Gaurilcikiene *et al.*, 2013]. When the exposure time was extended, there was an improvement in the activities of the enzymes in the seedlings. In our study, the activity of CAT and SOD increased with increases in the microwave power and treatment time. Chen *et al*. [2009a] reported that α-amylase activity in the seedlings first increased then decreased as the seed surface temperature increased when the wheat seeds were exposed to microwave radiation for 5, 10, 15, 20 and 25 s, and the higher temperature harmed seed germination. The GR of lentil (*Lens culinaris*) seeds exposed to the microwave power of 450–730 W for 30 s was improved; however, this rate was inhibited for longer exposure times (60 and 90 s) [Aladjadjiyan, 2010]. A lower power microwave pretreatment increased both POD and SOD activities and increased the GR and growth vigor [Chen *et al.*, 2009a], but a higher power and longer duration caused more negative effects on wheat seeds. Thus, a low power microwave treatment for a short time improved the GR, but a low power for a longer time slightly affected the GR; however, the GR was inhibited when the seeds were incubated for longer times at high power levels.

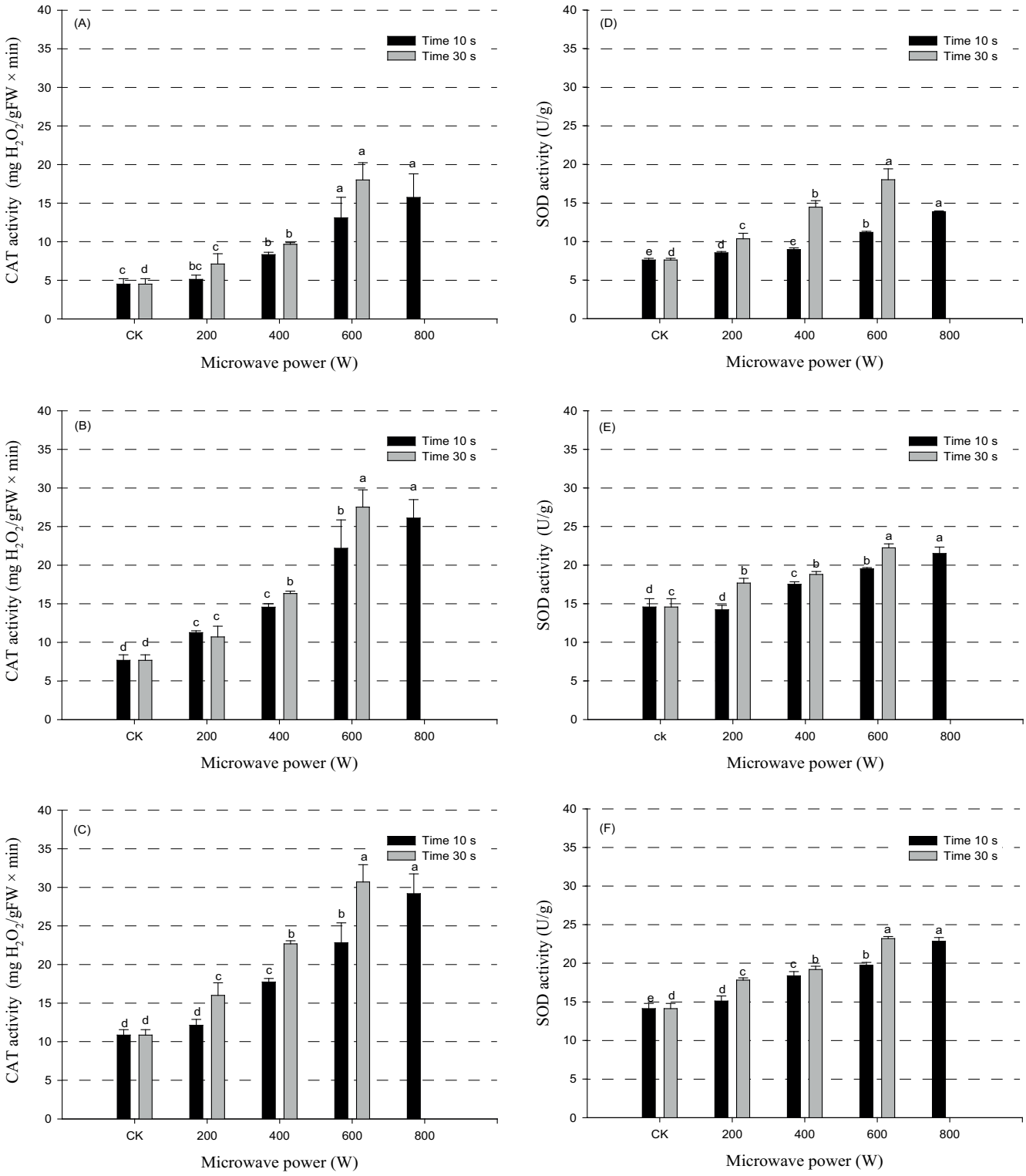


FIGURE 3. Changes in the catalase and superoxide dismutase activity of tartary buckwheat sprouts with different microwave treatments.

(A), (D): 3-day sprout; (B), (E): 5-day sprout; (C), (F): 7-day sprout. All values are the means±SD (n=50). Values of the catalase and superoxide dismutase activity with different microwave power levels shown with different lowercase letters are significantly different by ANOVA with Duncan's multiple range test at $P<0.05$. CK is the control. Seeds that were treated with microwaves at 800 W for 30 s had the lowest GR. The data of the test for the CAT and SOD activity is not shown.

Some non-invasive techniques such as blue light-UV [Ji *et al.*, 2016] and microwaves are being used to increase the yield of biologically-active components. These non-thermal effects on seeds and biological objects are due to the direct interaction of the microwaves with molecules or tissue components since particles seek to orient themselves within an electric field and minimize the potential energy [Radzevičius *et al.*, 2013]. Ji *et al*. [2016] reported that the total flavonoid content in the tartary buckwheat sprouts irradiated with blue light followed by UV-C (BL+UV-C) increased by 10% compared with

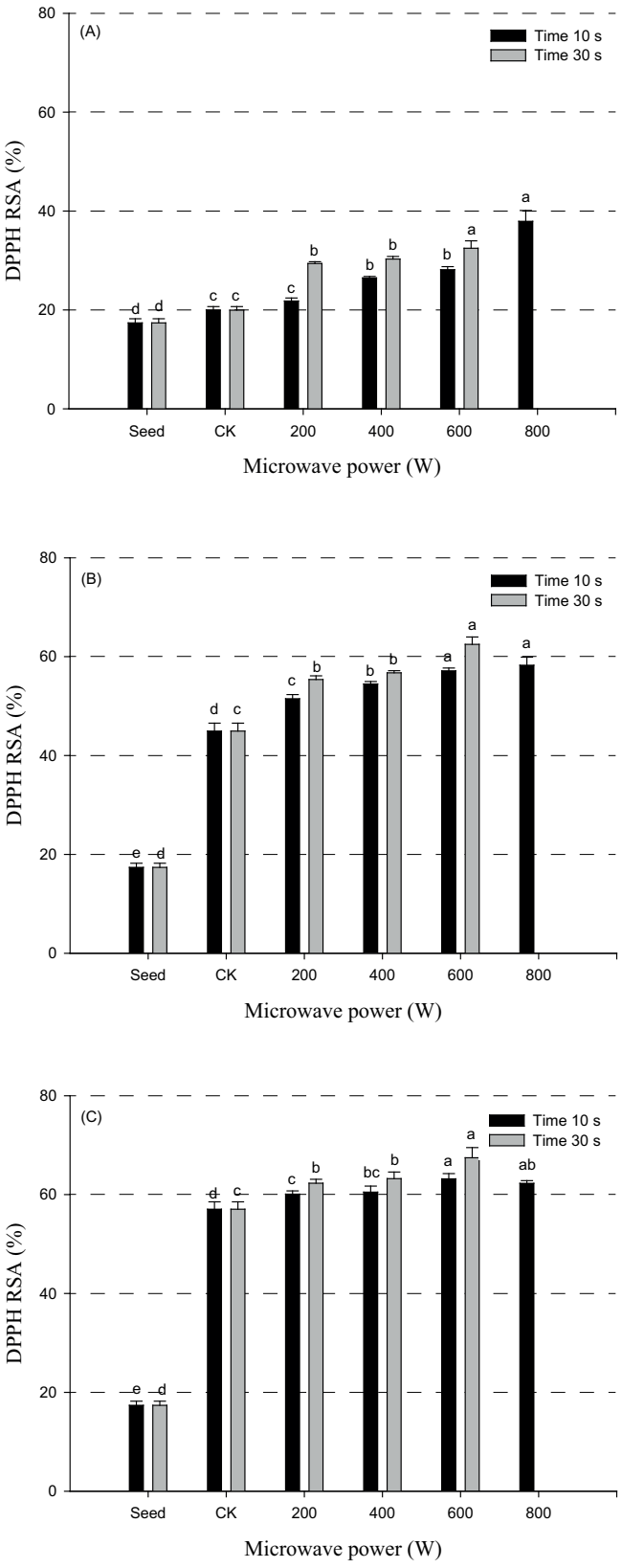


FIGURE 4. Changes in the DPPH radical scavenging activity of tartary buckwheat sprouts with different microwave treatments.

(A) 3-day sprout; (B) 5-day sprout; (C) 7-day sprout. All values are the means±SD (n=50). Values of the DPPH RSA with different microwave power levels shown with different lowercase letters are significantly different by ANOVA with Duncan's multiple range test at $P<0.05$. CK is the control. Seeds that were treated with microwaves at 800 W for 30 s had the lowest GR. The data of the test for the DPPH RSA is not shown.

the reverse sequence (UV-C+BL). Various light-emitting diodes (LEDs) had an influence on the accumulation of phenolic compounds in tartary buckwheat sprouts [Seo *et al.*, 2015]. The results indicated that microwave pretreatment increased some enzyme activities, GR and growth vigor [Chen *et al.*, 2009a]. However, key enzymes (PAL, CHI and rutin degrading enzymes) were revealed to have a significant correlation with total flavonoids in tartary buckwheat sprouts [Ji *et al.*, 2016]. Orsak *et al*. [2001] reported that microwave irradiation did not affect total polyphenols, but an increase in rutin increase was observed in some buckwheat samples. An increase of 78% was observed in the ascorbic acid content of the dry matter of potato tubers after microwave irradiation (P=90 W, 0 – control, 200 and 400 s) [Orsak *et al.*, 2001]. Zhou *et al.* [2015b] revealed that the total flavonoid content increased with an increase in the germination time of seeds. In our study, at the same treatment time, the total flavonoid content of sprouts first increased then decreased as microwave power increased. Under gentle microwave power, the total flavonoid content of sprouts increased as the exposure time increased.

In our study, at the same microwave power, the reducing sugar content of sprouts was higher at 30 s than 10 s. Moreover, an increase in the microwave power increased the reducing sugar content of the sprouts when the seeds were exposed to microwaves for the same time. Germination can increase the soluble protein content of sprouts, which first increased and then decreased from 3–7 days after exposure. To some degree, a longer exposure time and higher microwave power increased the soluble protein content in tartary buckwheat sprouts. In a previous study, a decrease in the total protein and the total sugar content in germinated seeds was accompanied by an increase in amino acids and reducing sugars, respectively [Zhou *et al.*, 2015b]. Microwave irradiation (P=90 W, 0 – control, 200 and 400 s) caused a decrease in contents of all acids investigated including the phenolic amino acid L-tyrosine (L-Tyr), chlorogenic acid (CGA), caffeic acid (CA), and ferulic acid (FA) in three buckwheat samples (seeds, seedlings and plants: *Fesculentum Moench*, cv. *Pyra* and *Emka* and *Tartarian buckwheat F. tataricum Gärtner*) [Orsak *et al.*, 2001]. Zhou *et al*. [2015b] demonstrated that the fatty acid contents showed no regular change trend with germination time for tartary buckwheat sprouts. Wheat (*Triticum aestivum L.*) grains were exposed to microwave radiation of wavelength 2.85 cm and a frequency of 10.525 GHz for 15, 45, or 75 min. The exposed grains were germinated and harvested after 7 and 14 days. The results showed that the microwave radiation increased the protein and amino acid content in wheat seedlings [Hamada, 2007]. Our study results concurred with those of Hamada *et al*. [2007]. At the same microwave power, the free amino acid content was reduced when the treatment time was extended from 10 s to 30 s. The free amino acid content of sprouts increased with an increase of microwave power, and first increased and then decreased in 5-day seedlings.

The antioxidant activity in seeds and sprouts is associated not only with the content of active ingredients but also with the types of active ingredients, including VC, vitamin E (VE), flavonoids, and carotenoids [Pasko *et al.*, 2009]. Tartary buckwheat is rich in flavonoids [Li *et al.*, 2016; Nam

et al., 2015]. Lee *et al*. [2016] indicated that the antioxidant activity is associated with the flavonoid content, and different flavonoids contribute differently to the total antioxidant activity of common and tartary buckwheat. In our study, the effect of microwaves on the DPPH RSA of sprouts had a similar trend on the total flavonoid content. Some previous studies indicated that rutin had the highest RSA of various phenolic compounds and exhibited the strong DPPH RSA. Therefore, the DPPH RSA was greatly affected by rutin content [Ji *et al.*, 2016]. A biochemical radical scavenging assay and gene expression results showed that the phenolic compounds in tartary buckwheat sprouts increased with a concomitant increase in the DPPH RSA upon exposure to L-phenylalanine (L-Phe) and LED lights [Seo *et al.*, 2015], and cold stress [Li *et al.*, 2015]. In our study, at the same microwave power, the DPPH RSA after a 30-s exposure was higher than after a 10-s exposure. At the same exposure time, an increase in the microwave power significantly increased the DPPH RSA. The DPPH RSA in sprouts increased when the seeds were germinated and as the germination time increased. Zhou *et al*. [2015a] reported a good accumulation in the total flavonoid content (approximately 20 mg rutin/g), and rutin (11 mg/g) was found after 7 days of germination, and germination improved the activities of buckwheat extract to scavenge DPPH by 107%. Furthermore, a correlation and principal component analysis showed that the total flavonoids and rutin content were closely and positively related to the free radical scavenging activity.

Thus, an increase in the microwave power significantly increased ($P<0.05$) the GR of tartary buckwheat seeds. The GR of tartary buckwheat seeds were significantly reduced when the treatment time was increased from 10 s to 30 s at a higher microwave power. At a power 800 W, the GR of the seeds was at a minimum and uniform germination was observed. However, gentle power (200 to 600 W) for a short time (10 s), increased the content of total flavonoids, reducing sugars, soluble proteins and free amino acids as well as the DPPH RSA of sprouts with an increase in microwave power. However, the above mentioned parameters decreased when the power exceeded 600 W. At the same power, the increase in exposure time from 10 s to 30 s increased the total flavonoid content, reducing sugar content and the DPPH RSA of the sprouts. However, as the microwave power was increased from 600 to 800 W, the total soluble sugar content had an opposite trend. With a gentle microwave power treatment, extending the treatment time helped increase the total flavonoids and reducing sugar content of the sprouts after the seeds were germinated. Microwave pretreatment not only had a short-term effect on seed germination but also a long-term effect on seed germination capability, seedling development, metabolism and biomass compared with the control. The effects of microwave field patterns, power and time on other key enzyme activities and the composition of tartary buckwheat were not investigated in the present study but will be the subject of a follow-up study.

Moreover, due to variations in the material mass and moisture content and properties, hot spots can form in the different microwave cavity, which can lead to uneven energy absorption and heating during microwave irradiation. Therefore, the effect of variations in mass and moisture content of wet material on the results should be considered with microwave irradiation. Unfortunately, the above problem has not been involved in this study.

CONCLUSIONS

Microwave treatment can increase the GR of the tartary buckwheat seeds. In summary, low-power and short exposure to microwaves can increase the GR of seeds; however, low power for a long time also affected seed germination, while exposure to a high power for a long time inhibited seed germination. When the seeds were treated with an appropriate dose of microwave irradiation and time, the microwave treatment increased the total flavonoid content, the soluble protein content, the reducing sugar content, the free amino acid content, and the activities of CAT and SOD in sprouts, thus improving the DPPH RSA.

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CONFLICT OF INTERESTS

Authors declare no conflict of interest.

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