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Full Length Research Paper

Toward a novel understanding of buckwheat self-defensive strategies during seed germination and preliminary investigation on the potential pharmacological application of its malting products

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Common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat (*Fagopyrum tataricum* L. Gaertn) were used as materials to study plant self-defensive strategies against oxidative stress and pathogen infection during seed germination. Sprout development induced hull structure split, membrane degradation and tissue injury, accompanied with production of a large amount of reactive oxygen species (ROS) such as superoxide (O_2^-), hydroxyl radical (·OH), alkoxyl radical (R·) and hydrogen peroxide (H_2O_2). Therefore, buckwheat sprouts had to face both oxidative stress and environment pathogen aggression during the course of malting. To analyze the antioxidant capacity of ROS by buckwheat seeds, the activity of four kinds of antioxidant enzyme was studied, including superoxide dismutase, catalase, peroxidase and ascorbate peroxidase. Scavenging effects of 2, 2-diphenyl-1-picrylhydrazyl free radicals were also investigated. Both gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*, *Salmonella typhimurium* and *Bacillus subtilis*) were applied to detect antibacterial activity of the germinated seeds against microorganism pathogen infection. Ethanolic extracts of flavonoids from germinated seeds revealed an obvious anti-tumor activity against human lung carcinoma cell line by MTT assay. Our research in this paper indicated that through long term evolution, buckwheat had already developed advanced strategies to repair inner injury and to resist the outer environmental risks during seed germination. The enriched production of flavonoids in the germinating stage might quite possibly be applied in clinical field to deal with lung cancer.

Key words: Common buckwheat, tartary buckwheat, germinated sprout, antioxidant activity, antibacterial activity, antitumor activity, lung cancer.

INTRODUCTION

Buckwheat is a kind of cereal-like crop mainly grown in mountain and plateau area, where soil is barren with less water, rare nutrition and low temperature. It is usually believed that cultivated common buckwheat originated in Sanjiang area of China (Konishi et al., 2005). Buckwheat is also wildly cultivated in Japan, Korea, Europe and North America. However, three most popular buckwheat cultivars are separately *Fagopyrum esculentum* Moench, *Fagopyrum tataricum* (L.) Gaertn and *Fagopyrum dibotrys* (D. Don) H. Hara. Buckwheat seed is rich in nutrients such as D-chiro-inositol, flavonoids, protein, vitamins and other unique components. Therefore, food made from

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Abbreviations: ROS, Reactive oxygen species; **SOD,** superoxide dismutase; **CAT,** catalase; **POD,** peroxidase; **ASP,** ascorbate peroxidase; **2, DPPH•,** 2-diphenyl-1-picrylhydrazyl; **BWAEs,** buckwheat antioxidant enzymes; **BWFEs,** buckwheat flavonoid extracts.

buckwheat flour has been considered as potential therapy to treat various chronic diseases, such as diabetes, hypercholesterolemia, hypertension, cardiovascular disorder (Li and Zhang, 2001).

The nutritional value of many kinds of grain seeds can be elevated by germination. However, during the course of germination, simultaneously with hull structure split, membrane degraded and tissue injured, buckwheat seed falls in a dangerous condition because of both noxious physical factor (for example, reactive oxygen species, ROS) and environmental microorganisms. Buckwheat successfully overcome this problem by constructing biological defense system, consisting of flavonoids (Oomah and Mazza, 1996), antimicrobial peptides (Fujimura et al., 2003) and protease inhibitor (Park et al., 1997; Khadeeva et al., 2009).

Oxidation induced by ROS can cause serious damage or disorder in plants, animals, and even humans. Therefore, a large quantity of researches has been done to explore antioxidants in natural lives, including buckwheat, amphibian and tick (Oomah and Mazza, 1996; Yang et al., 2009; Wu et al., 2010). Through analyzing the mechanism of antioxidant activities occurring in natural lives, various antioxidants are investigated at the aim of treating human diseases, for example, cancer, liver disease, aging, arthritis, inflammation, diabetes, Parkinson's disease, atherosclerosis, and AIDS (Moom and Shibamoto, 2009). Buckwheat is well-known for its abundant in antioxidant components, consisting of flavonoids (Oomah and Mazza, 1996), enzymes (Jovanovic et al., 2006), peptides (Ma et al., 2010), and phenolic constituents (van den Berg et al., 2008). Researches on buckwheat antioxidant activity mainly focus on scavenging of free radicals or anions and inhibition of lipid peroxidation (Oomah and Mazza, 1996; Ma et al., 2010; van den Berg et al., 2008), whereas activity of antioxidative enzymes in buckwheat sprouts is not much cared for. Therefore, we investigated the activity of antioxidant enzyme, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (ASP), as well as the scavenging of free radicals DPPH• .

Plant oxidative stress may be partially induced by pathogens (Amari et al., 2007), which caused harmful damage to wounded tissue during seed maturation. Various beneficial compounds discovered from buckwheat are found to have antibacterial activity, including protease inhibitor (Khadeeva, 2009), antibacterial protein (Valcu et al., 2009), antibacterial peptides (Fujimura et al., 2003; Leung and Ng, 2007), tannins (Amarowicz et al., 2008), phenolic constituents (van den Berg et al., 2008), and flavonoids (Fabjan et al., 2003). This defensive system has a very critical meaning for embryo safety against microorganism infection during germinating phase. Therefore, the antibacterial activity of buckwheat total flavonoid extracts was examined. Furthermore, to study other anti-inflammatory property of buckwheat ethanolic extracts and their potential healing use, antiproliferative activity of human lung cancer cell A549 were also tested.

MATERIALS AND METHODS

Two species of buckwheat (common buckwheat *F. esculentum* Moench. and tartary buckwheat *F. tataricum* Gaertn) were cultivated in Shanxi province of China in 2007. Till the harvested festival, the seeds were collected and dried. The human lung carcinoma cell line A549 was purchased from cell bank in Shanghai institutes for biological sciences of Chinese Academy of Sciences. Four strains of gram-positive and -negative bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Bacillus subtilis*) were cultured in our laboratory.

Malting of buckwheat seeds

Buckwheat seeds were cleaned and washed in 0.9% (w/v) NaCl solution, and then marinated in deionized water for 10 h. The soaked seeds were put into 8-layers sterilized gauze, and malted for 0 to 7 days in SPX-300 IC microcomputer artificial climate chamber (30°C, 80% humidity). The malted sprouts were dehulled and dried by lyophilization, and then crushed into powder.

Preparation of crude enzyme solutions

The crude antioxidant enzyme solution was extracted and referred to Wang et al. (1983) with certain modification. The malted powder of buckwheat seeds (already described in this paper) was dissolved in phosphate balanced solution (PBS, 50 M, pH 7.8), added with 0.1 M EDTA and 4% (w/v) phosphate-pyrrolidone (PVP). The enzyme extract solution was adequately rubbed on the ice with quartz sand added, and then centrifuged at 6500 g for 30 min. The supernatant of antioxidant enzyme was collected and stored at -20°C for later use.

Superoxide dismutase (SOD) activity assay

The SOD activity assay of crude enzyme solution was carried out according to the procedure provided by Genmed coporation (USA). Briefly, the reaction system (200 µl staining working solution, 20 µl Genmed catalyze working solution and 20 µl crude enzyme solution) was incubated in dark at 37°C for 20 min, then absorbance was detected at 450 nm. Enzyme activity of SOD was expressed by inhibition rate of 50% of the protein concentration (IC 50).

Catalase (CAT) activity assay

Activity of CAT was assayed as described by Jovanovic et al. (2006) and Himelbloom and Hassan, (1986) with little change. Generally, 3 ml reaction system consisted of the following chemicals: 40 µl crude enzyme solution or blank control, 300 µl H_2O_2 (0.1 M), 1.66 ml phosphate balanced solution (PBS, 0.2 M, pH 7.8), and 1 ml deionized water. After H_2O_2 was added, absorbance at 240 nm (A_{240}) was detected. CAT activity was normalized by decrease of A_{240} per minute per mg of protein.

Peroxidase (POD) activity assay

Activity of POD was determined as described by Abbott et al. (1984). The reaction mixture was added as following: 2.9 ml PBS (0.2 M, pH 6.0), 1 ml H_2O_2, 1 ml guaiacol, and 100 µl crude enzyme solution or

blank control. The reaction was originated at 37°C, with absorbance at 470 nm (A_{470}) assayed. Enzyme activity of POD was revealed as $\Delta A_{470}\,min^{-1}$ (mg of protein)$^{-1}$.

Ascorbate peroxidase (ASP) activity assay

Activity of ASP was examined as stated by Dalton et al. (1987). Total 3 ml of reaction mixture consisted of 50 M PBS (pH 7.0), 0.1 mM EDTA-Na_2, 0.3 mM AsA, 10 mM H_2O_2 and 100 µl crude enzyme solutions. Absorbance at 290 nm (A_{290}) was recorded every 10 s as reaction initiated. The ASP activity was expressed as ΔA_{290} min^{-1} (mg of protein)$^{-1}$.

Scavenging of 2.2-diphenyl-1-picrylhydrazyl (DPPH•) free radicals

The scavenging of DPPH• referred to the method described by Cotelle et al. (1996) .Concisely, total 4 ml of reaction mixture including 3 ml DPPH• solution (100 µM), 1 ml crude enzyme solution or blank control, mixed completely and shelved for 30 min. Absorbance at 517 nm of blank control (A_0) and sample (A_s) were assayed, and the scavenging rate was calculated as following formula:

Scavenging rate (%) = $(A_0 - A_s)/ A_0 \times 100\%$.

Ethanolic extract of flavonoids from malting buckwheat seeds and its antibacterial activity

The powder from malted buckwheat seeds (already described) was dissolved in 70% ethanol for flavonoid extraction. After extracting in 70°C water bath for 6 h, the extraction mixture was filtered, and the filtrate was stored for further use (Watanabe et al., 1997).

The antibacterial activity method was referred to previous research (Khadeeva et al., 2009; Lai et al., 2004; Shih, 2009). Four strains of gram-positive and -negative bacteria (*S. aureus, E. coli, S. typhimurium* and *B. subtilis*) were grown in LB broth with 90 mm Petri dish containing 25 ml of 1.5% agar. The test sample was added on a circular filter paper (diameter 6 mm) and dried, and then laid onto the top of agar. After incubation overnight at 37°C, antimicrobial zone was observed. The outside diameter of antimicrobial zone (o.d.) and the inside diameter of filter paper (i.d.) were measured, and annular width (Δx) revealing antibacterial ability were calculated as the result of (o.d.) minus (i.d.). Untreated filter paper was used as blank control, while 100 U/ml antibiotics (penicillin) was used as positive control.

Cell culture and MTT assay

Human lung carcinoma cell line A549 was cultured in RPMI-1640 medium (37°C, 5% CO_2), containing 10% (v/v) fetal calf serum (FCS) and supplemented with 100 U/mL penicillin, 100 mg/ml streptomycin. Culture medium was replaced every 2 to 3 days. After cells grew to near confluence, they were treated with 0.25% (w/v) trypsin and 0.02% (w/v) EDTA for 5 min and replaced to a new flask.

The anti-proliferation assay of A549 was measured with MTT assay (Mosmann, 1983; Hongrapipat et al., 2008). A549 cell line (2.5×10^4 cells) were transferred to 96-well microtiter plates, after 48 h incubation, 10 µl of 0.5% (w/v) MTT solution was added to each as well. After dying for 4 h at 37 C in a 5% CO_2 environment, absorbance was measured with a microtiter plate reader (Tacan GENios Pro, Germany) at 570 nm. All experiments were carried out in three independent repeats, and the 50% inhibitory concentration (IC_{50}) was detected.

Statistical analysis

The statistical analysis of the data was carried out by SPSS 13.0 statistical software. The results were expressed as the mean ±SEM of at least three experiments. While comparing the change, the data was analyzed by one-way ANOVA, followed by the LSD to detect significant difference between different groups. The level of significance was set at $P<0.05$.

RESULTS

Extraction of crude enzyme solutions

The crude antioxidant enzyme solution extracted from malted powder of buckwheat seeds (day 0 to 7) were collected. The different extracts were separately characterized by the day of germination. Therefore, antioxidant enzyme solutions from common buckwheat (*F. esculentum Moench.*) sprouts were named as buckwheat antioxidant enzymes (BWAE-C0-7) for short. Simultaneously, antioxidant enzyme solutions from tartary buckwheat (*F. tataricum Gaertn.*) sprouts were named as buckwheat antioxidant enzymes (BWAE-T0-7) for short.

Enzyme activity of superoxide dismutase (SOD)

The changes of SOD activity in buckwheat seeds during germination are shown in Figure 1. Apparently, SOD activity was enhanced in both common buckwheat and tartary buckwheat. The variation trends of SOD activity in two species of buckwheat were surprisingly similar. That is, enzyme activities rised continuously from the 0 to 5th day and then degraded rapidly during the 6-7th day. The climax of enzyme activity presented on the 5th day (Figure 1), therefore the enzyme activity BWAE-C5 and BWAE-C5 reached 824.521 U/mg and 608.349 U/mg respectively. Generally, SOD activity of tartary buckwheat was higher than that of common buckwheat.

Enzyme activity of catalase (CAT)

The changes of CAT activity in buckwheat seeds during germination are revealed in Figure 2. We could find that the variation trends of CAT activity in both common buckwheat and tartary buckwheat were nearly the same, with two times of degradation and two times of enhancement. During early stage of germination (the 0 to 2 day), CAT activity went down to the lowest ebb, especially in tartary buckwheat. Later, CAT activity emerged a temporary rise on the 3rd day. After the secondary decrease on the 4th day, the activity of CAT

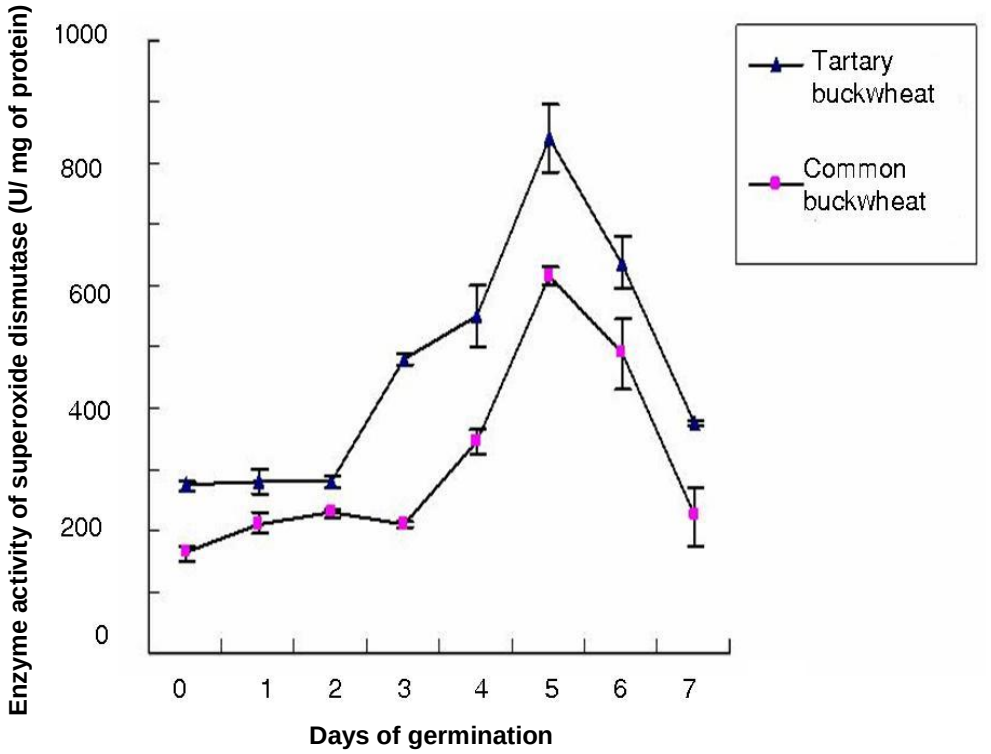

Figure 1. Comparison of superoxide dismutase (SOD) activity in common buckwheat and tartary buckwheat during seed germination. All the data demonstrated are the mean of three independent experiments.

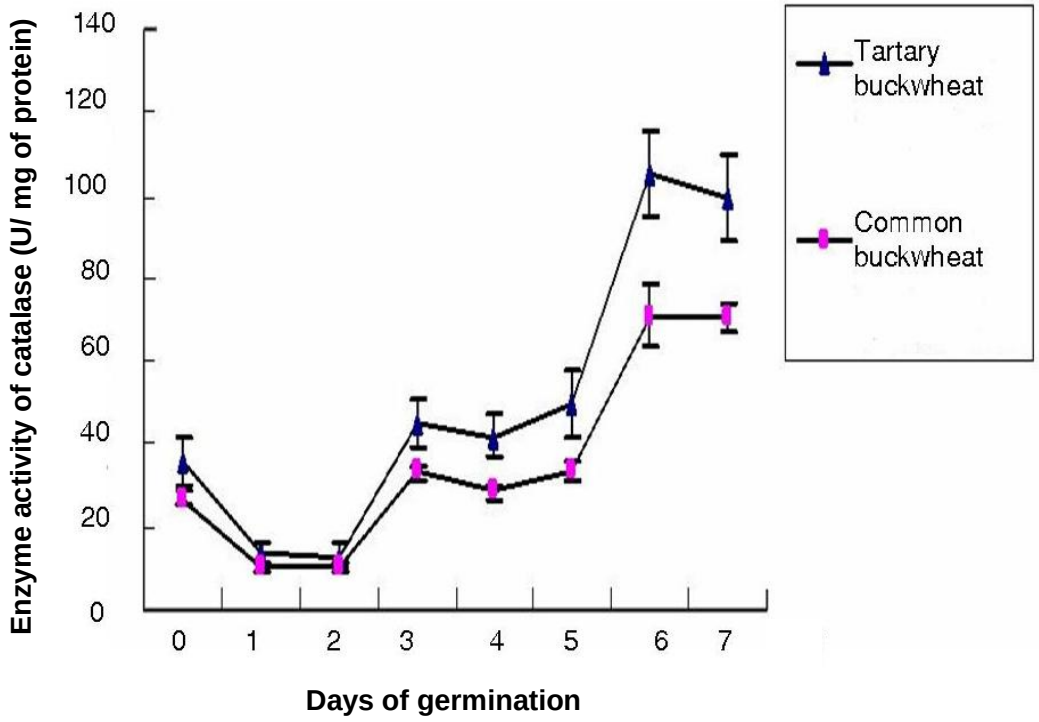

Figure 2. Comparison of catalase (CAT) activity in common buckwheat and tartary buckwheat during seed germination. All the data demonstrated are the mean of three independent experiments.

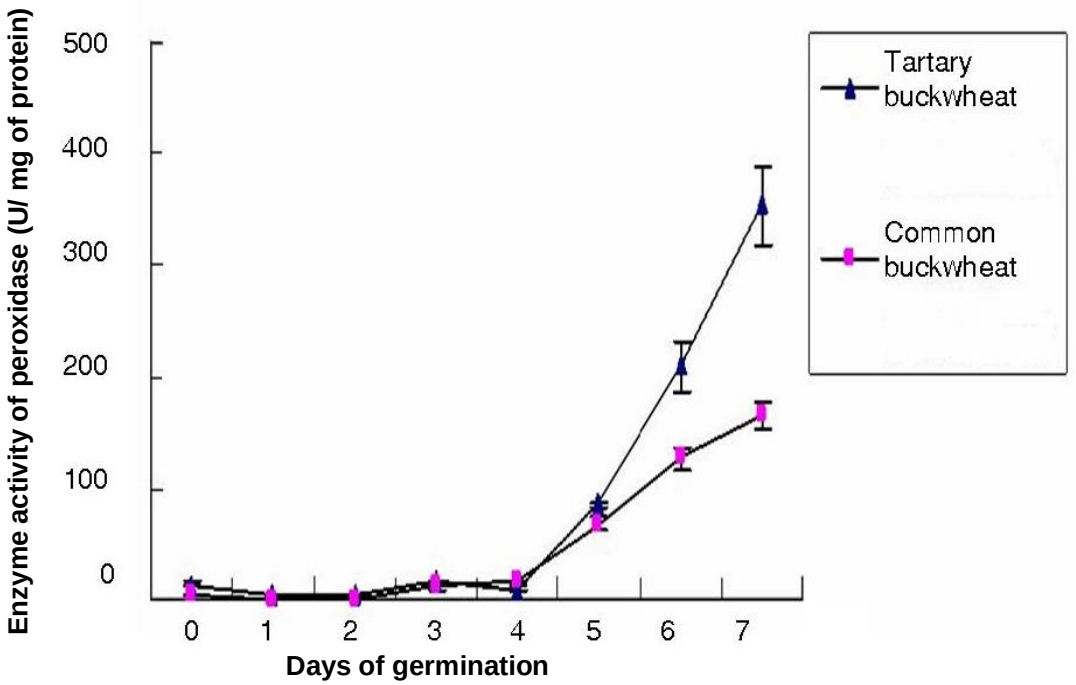

Figure 3. Comparison of peroxidase (POD) activity in common buckwheat and tartary buckwheat during seed germination. All the data demonstrated are the mean of three independent experiments.

increased promptly in the last three days.

Enzyme activity of peroxidase (POD)

Variations of POD activity were exhibited in Figure 3. Contrary to our anticipation, the enzyme activities of both common buckwheat and tartary buckwheat did not alter for the initiating period (on the 0 to 4 day), however, the enzyme activities of POD continuously ascend after the 5th day, and reach the top on the 7th day.

Enzyme activity of ascorbate peroxidase (ASP)

Different from the aforementioned 3 species of antioxidant enzymes, the activity of ASP did not alter obviously during germination on the 7th day (Figure 4). As to tartary buckwheat, the curvilinear change of ASP activity rose slightly on the 1, 3 and 5th day. For common buckwheat, there were only two times of promotion of the activity of ASP on the 3 and 5th day.

Scavenging capacity of 2.2-diphenyl-1-picrylhydrazyl (DPPH•) free radicals

As shown in Figure 5, the scavenging actions of 2.2-diphenyl-1-picrylhydrazyl (DPPH•) by antioxidant enzymes solution from common buckwheat and tartary buckwheat were effective. The elimination properties of DPPH• free radicals of both common buckwheat and tartary buckwheat seeds were augmented slightly by the malting process.

Ethanolic extract of flavonoids from malting buckwheat seeds and its antibacterial activity

The buckwheat flavonoid extracts (BWFEs) from malted seeds (the 0 to 7th day) powder were collected separately during germination, according to different phases of germination, flavonoid extracts were designated as BWFE-C0-7 (from common buckwheat) and BWFE-T0-7 (from tartary buckwheat). BWFEs were tested for antibacterial activity versus 4 strains of gram-positive and -negative bacteria (*S. aureus*, *E. coli*, *S. typhimurium* and *B. subtilis*), and the annular width (Δx) expressing antibacterial ability was measured and calculated. As shown in Table 1, almost all the tested samples demonstrated certain degree of antibacterial activity. The effects of BWFEs towards *S. typhimurium* and *B. subtilis* were much better than that on *S. aureus,* while the inhibitory actions on *E. coli* were the weakest.

Cells proliferation inhibitory effects by MTT assay

The antitumor action of buckwheat flavonoid extracts (BWFEs) on human lung cancer cell line A549 was

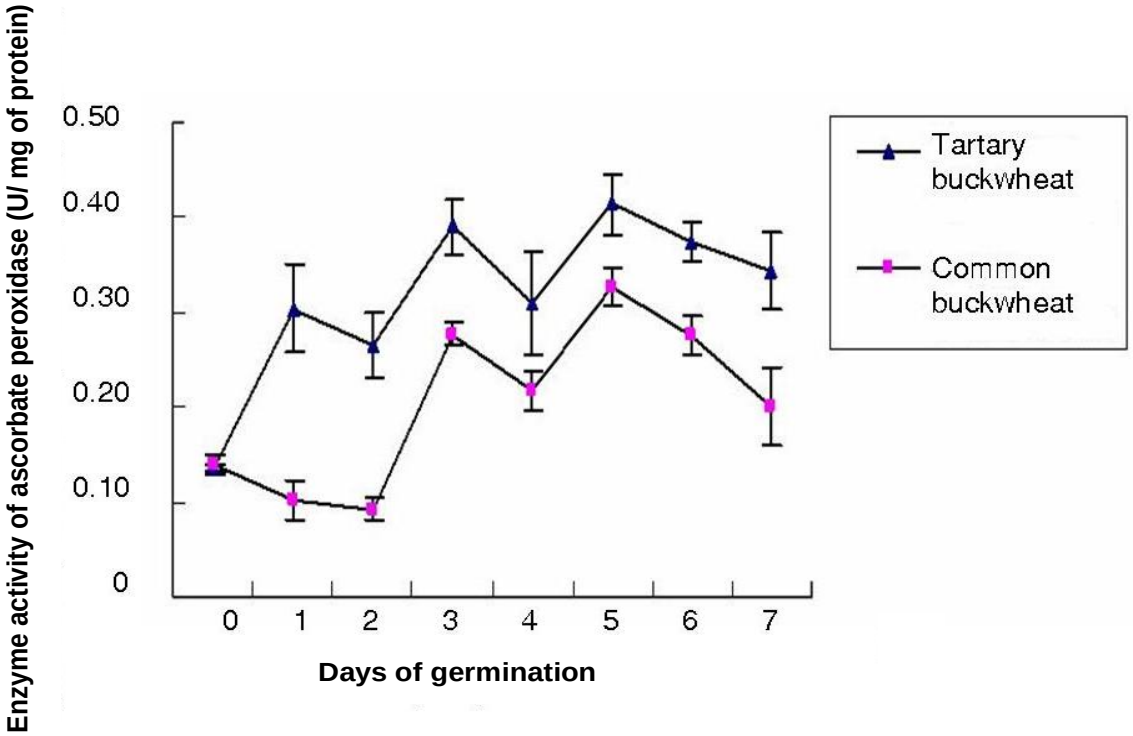

Figure 4. Comparison of ascorbate peroxidase (ASP) activity in common buckwheat and tartary buckwheat during seed germination. All the data demonstrated are the mean of three independent experiments.

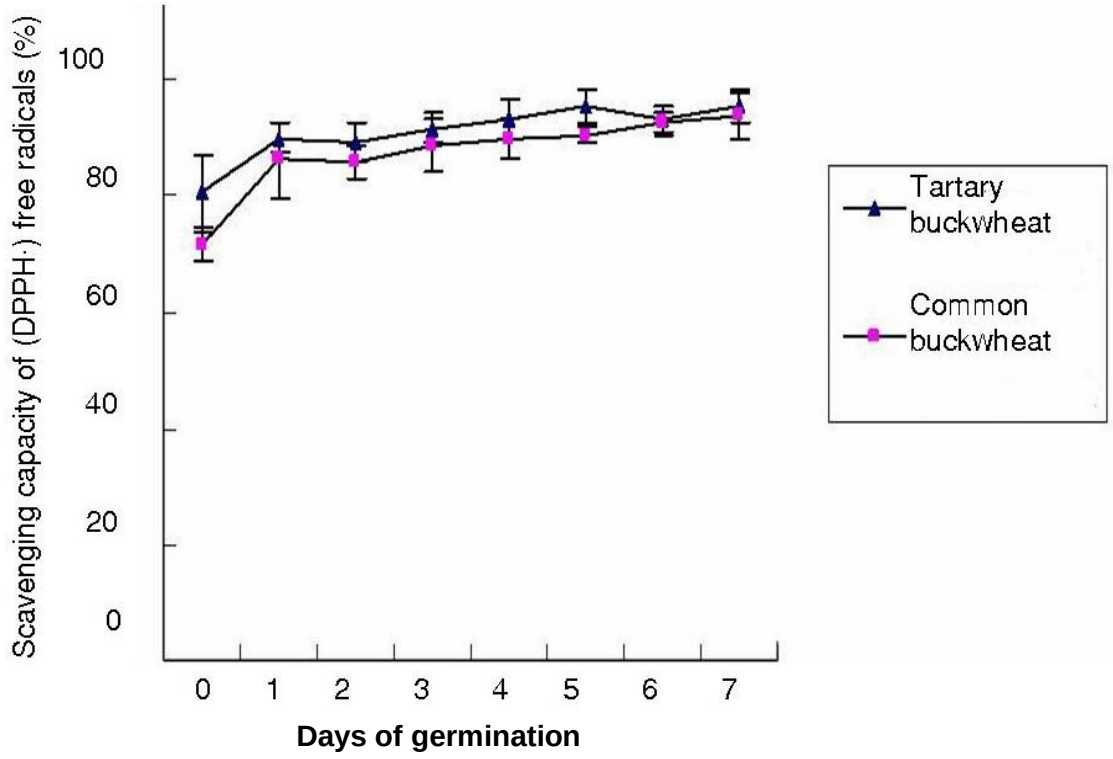

Figure 5. The scavenging effects of DPPH• free radicals by antioxidant enzyme solutions from common buckwheat and tartary buckwheat. All the data demonstrated are the mean of three independent experiments.

Table 1. Antibacterial activity study of flavonoid extracts from malting buckwheat seeds.

Favonoids extracts	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhimurium*	*Bacillus subtilis*
Blank	0.0	0.0	0.0	0.0
Positive control	6.3±2.1	6.4±1.8	8.0±1.0	5.0±1.0
BWFE-C0	2.5±0.3	3.0±0.7	4.4±1.1	3.1±0.2
BWFE-C1	2.3±0.4	1.7±0.4	2.8±0.7	4.2±0.4
BWFE-C2	3.2±0.6	2.4±1.5	3.1±0.9	4.2±0.4
BWFE-C3	3.6±1.2	1.8±0.3	4.3±0.3	4.3±0.4
BWFE-C5	3.8±1.0	1.5±0.5	3.7±0.8	2.1±0.7
BWFE-C6	3.7±0.6	1.5±0.5	4.2±0.7	3.6±0.9
BWFE-C7	2.9±0.8	1.5±0.4	3.3±0.7	3.4±0.5
BWFE-T0	3.0±0.5	2.3±1.1	3.8±0.7	3.6±0.5
BWFE-T1	3.2±0.7	2.5±0.6	3.6±0.2	4.5±0.9
BWFE-T2	3.2±1.2	2.2±0.4	3.1±0.7	4.8±1.5
BWFE-T3	4.1±0.2	1.8±0.8	3.2±0.7	2.7±0.8
BWFE-T4	3.0±0.9	2.7±1.1	4.4±0.9	4.2±0.8
BWFE-T5	3.8±1.2	1.9±0.4	2.8±0.3	1.8±0.4
BWFE-T6	2.8±1.2	2.0±1.2	4.8±1.3	2.8±0.8
BWFE-T7	3.6±0.4	3.1±0.7	4.3±1.3	5.0±1.5

Annular width (Δx, mm) expressing antibacterial ability was calculated as outer diameter (o.d.) minus inside diameter (i.d.).

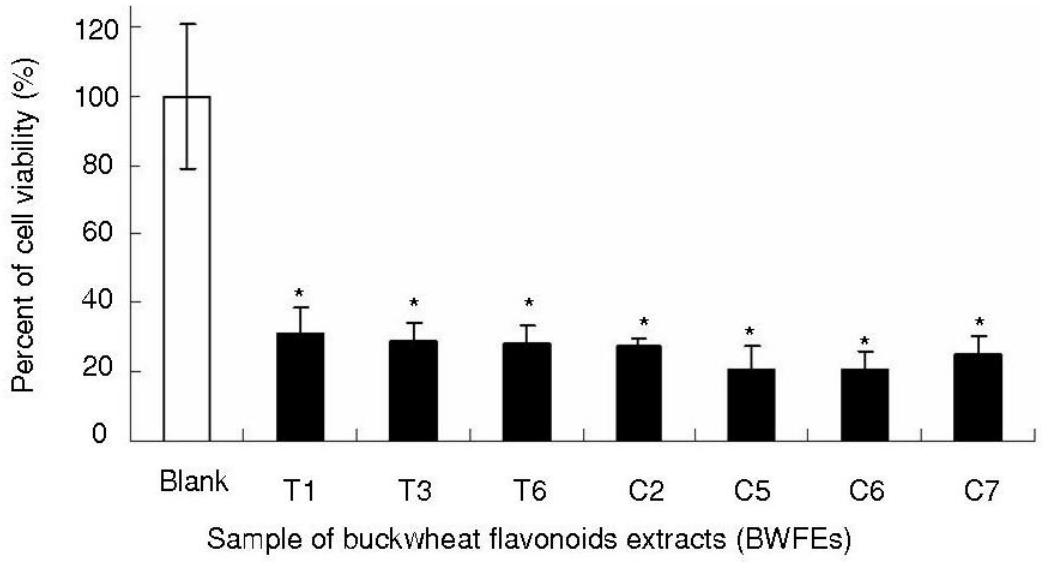

Figure 6. Cells proliferation inhibitory effects of BWFEs against lung cancer cells A549. Blank group with no sample added, and tested group was added with different buckwheat flavonoid extracts. *: with significant difference (p<0.01). All the data shown were obtained from three independent experiments.

examined, using a modified MTT assay. Seven tested samples exhibited a significant inhibitory effect (Figure 6) on A549 cell proliferation, including BWFE-T1, 3 and 6 from tartary buckwheat, and BWFE-C2, 5, 6 and 7 from common buckwheat. Compared with untreated cells in blank control wells, the cell viability of treated cells was greatly inhibited (p<0.001, compared with blank control). Furthermore, the values of IC50 were obtained as shown in Table 2.

DISCUSSION

Seed germination process may induce many physical and

Table 2. IC50 (50% inhibitory concentration) of BWFEs inhibited cell proliferation on A549 cancer cell line.

Favonoid extracts	IC50 values (μg/ml)
BWFE-T1	24.69±0.59
BWFE-T3	10.89±0.14
BWFE-T6	31.59±2.7
BWFE-C2	184.61±6.59
BWFE-C5	99.43±1.67
BWFE-C6	99.61±16.58
BWFE-C7	61.59±3.09

chemical changes in plant sprouts. On one hand, plant has to develop strategies to resist both the inner damages and outer pathogens invading threat (van den Berg et al., 2008; Amari et al., 2007). On the other hand, seed embryo must also prepare the increasing demands of growing seedling (Fabjanv et al., 2003; Lu et al., 2007). Therefore, level of various functional molecules (for example, antioxidative enzymes, antibacterial protein, antibacterial peptides, flavonoids, etc) in the sprout alters to adapt the survival stress occurring during seed germination (Lu et al., 2007; Nair and Adachi, 1999).

In this study, we employed two different methods to investigate the changes of antioxidative enzymes and scavenging capacity of free radicals in malted buckwheat seeds, and their anti-infection ability towards common microorganisms, as well as the potential application for functional food product exploring.

ROS such as superoxide anion O_2^- , hydroxyl radical •OH, alkoxyl radical RO• and hydrogen peroxide (H_2O_2) can cause membrane injury by lipid peroxidation (Moon and Shibamoto, 2009). It is commonly believed that antioxidative proteins (for example, antioxidative enzymes and iron-binding proteins) can inhibit the lipid peroxidation (Elias et al., 2008) hence we investigated the activity of antioxidative enzymes SOD, CAT, POD and ASP. According to our research in this paper, enzyme activities of SOD, CAT and POD are all activated, accompanied by the proceeding of malted time. At the early stage of germination (on the 0 to 3rd day), enzyme activities of both SOD and POD almost did not alter (Figures 1 and 3), indicating that the O_2^- in the seeds keep in a stable level. For the next 1 to 2 days, enzyme activities of both SOD and POD began to rise. However, till the late stage, the enzyme activity of POD kept rising and reached a maximum (Figure 3), while the enzyme activity of SOD got to the top on the 5th day, then dropped quickly on the 6 to 7th day (Figure 1). The enhanced enzyme activity may be related to the generation of free radicals by seed malting, whereas the dropping of SOD activity is really hard to understand. According to CAT (Figure 2), enzyme activity decreased for the first three days, and then increased rapidly in the later stage of the germination. This result matched well with the previous study of antioxidant activity alteration of barley (Lu et al., 2007). The activity of ASP ebbed and flew during 7 days of malting, but didn`t change obviously. Both tartary buckwheat and common buckwheat show nearly the same altering trends of antioxidant activities, including antioxidative enzyme activity and ROS scavenging property (Figure 5). So we can conclude that buckwheat has developed a well-organized and self-defense mechanism against endogenic radicals.

During seeds germination, various pathogens in the steeping surroundings play an important role in the production of oxdative stress (Amari et al., 2007; Chauhan et al., 2007), accompanied by hulls structure split and tissue injury. Through long term of natural selection, plant has succeeded in dealing with bacteria by building molecular barriers consisting of antibacterial peptides (Fujimura et al., 2007; Leung et al., 2007), tannins (Amarowicz et al., 2008), phenolic constituents (van den Berg et al., 2008), and flavonoids (Fabjan et al., 2003). Buckwheat flavonoid extracts (BWFEs) from malted seedlings (from 0 to 7th days) were collected and tested for study (Table 1). Surprisingly, almost all the tested samples demonstrated certain degree of antibacterial activity. The antibacterial ability against *S. typhimurium* and *B. subtilis* were much higher than that against *S. aureus.* Though, the inhibitory effects on *E. coli* were not remarkable, the antibacterial activity of *Escherichia coli is* worthy further studying, considering *E. coli* as a kind of foodborne microorganism.

Buckwheat has constructed self-protective systems to face with possible challenges emerging during seed germination. Since long time ago, people get to know improving food nutritional value by germination. We detected the antitumor activity of BWFEs. To our activity anticipation, we found seven samples revealing promising antitumor activity (Figure 6), and the values of IC50 were obtained as shown in Table 2. Therefore, we have demonstrated that BWFEs may become potential therapy to cure lung cancer.

Conclusions

During the germinating phase, buckwheat sprouts had to face with a series of survival challenge including hull structure split, membrane degraded and ROS accumulated. Our research in this paper proved that buckwheat had developed self-defensive strategies to scavenge free radicals by enhancing the activity of antioxidant enzymes, such as SOD, CAT and POD. Though the enzymes activity enhancement were separatedly evoked at different periods of germination from 0 to 7 day, the clearance of DPPH• free radicals were significantly agumented during germination. Soil microorgnism was also a great threat to germinated buckwheat sprouts, since partial tissue was injured by malting. However, the flavonoid ingredients of buckwheat

seed revealed an obvious antibacterial activity against both gram-positive bacteria (*S. aureus*) and gram-negative bacterias (*E. coli*, *S. typhimurium* and *B. subtilis*). Interestingly, flavonoid extracts of buckwheat seeds also demonstrated antitumor property against human lung carcinoma cell line A549, therefore, the malted product of buckwheat seed could be explored as functional food additives to treat lung cancer.

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