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Improving the antioxidant activity of buckwheat (*Fagopyrum tataricm* Gaertn) sprout with trace element water

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Abstract

Trace element water (TEW) (100, 200, 300, 400 and 500 ppm) was used to grow buckwheat (*Fagopyrum tataricm* Gaertn) to evaluate whether the beneficial effects of trace elements on the antioxidant activity could be accomplished with the supplement of TEW. At 300 ppm, TEW significantly increased the Cu, Zn and Fe contents in buckwheat sprout, but not the Se and Mn contents. The levels of rutin, quercitrin and quercetin did not differ between buckwheat sprouts grown in TEW and de-ionized water (DIW). The ethanolic extract from buckwheat sprout grown in 300 ppm TEW showed higher DPPH radical scavenging activity, ferrous ion chelating activity, superoxide anion scavenging activity and inhibitory activity toward lipid peroxidation than that grown in DIW. The extract of the TEW group also enhanced intracellular superoxide dismutase activity and resulted in lower level of reactive oxygen species in human Hep G2 cells.
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Keywords: Buckwheat; *Fagopyrum tataricm* Gaertn; Trace element water; Antioxidant; Rutin

1. Introduction

Buckwheat is grown in many countries in Asia, Europe, South Africa, Canada, USA, Brazil and in certain other places around the world. The most popular derivative food is buckwheat noodles, very popular in Japan, China and Italy, made from buckwheat flour–water dough. The presence of rutin in buckwheat is one of the main reasons for the production of different kinds of buckwheat foods (Kreft, Fabjan, & Yasumoto, 2006). Among fruits, vegetables and grain crops, grapes and buckwheat are the most important rutin containing foods. No rutin was found in cereals and pseudocereals except buckwheat, which can be used as a good source of dietary rutin (Kreft, Knapp, &

Kreft, 1999; Park et al., 2000). Rutin is a secondary plant metabolite that antagonizes the increase of capillary fragility associated with haemorrhagic disease, reduces high blood pressure (Abeywardena & Head, 2001), decreases the permeability of the blood vessels and has an anti-oedema effect, reduces the risk of arteriosclerosis (Wojcicki, Barcew-Wiszniewska, Samochowiec, & Rozewicka, 1995) and has antioxidant activity (Park et al., 2000).

Trace element status is very important to maintain biological functions in all living organisms. Trace elements are involved in the antioxidant responses, inflammation, wound healing and immune responses (Prohaska & Luck-asewycz, 1981; Roussel, Anderson, & Favier, 2000). For the antioxidant system, several trace elements are involved in the main cellular defenses against reactive oxygen species (ROS), as co-factors of superoxide dismutase (SOD) or glutathione peroxidase (GPx) (Geret & Bebianno, 2004; Steinman, 1982; Wichtel, 1998). These enzymes protect cell

membranes from damage caused by the peroxidation of lipids. For example, manganese (Mn) plays a major antioxidant role, both as Mn-SOD co-factor and as a direct ROS scavenger. Zinc (Zn) can stimulate the activity of SOD. Selenium (Se) is an essential trace element for animals and humans because of its role in GPx (Tinggi, 2003). In this study, the effect of trace element water on the antioxidant activity of buckwheat sprout was determined. Measurement of antioxidant activities included reducing power, DPPH radical scavenging activity, ferrous ion chelating activity and inhibitory activity toward lipid peroxidation, as well as intracellular antioxidant enzyme activities, reactive oxygen species and superoxide anion in human Hep G2 cells.

2. Materials and methods

2.1. Materials

Buckwheat (*Fagopyrum tataricm* Gaertn) seed was obtained from Taichung District Agricultural Research and Extension Station, Council of Agriculture Executive Yuan, Taichung, Taiwan. Trace element water (TEW) was obtained from Shimanishi Kaken Co., Ltd. (Tokyo, Japan). Human hepatoma cell line (HepG2 cells) was obtained from Bioinformatics Lab., BCRC, Food Industry Research and Development Institute (Shinzu, Taiwan).

Rutin, quercetin, quercitrin, ascorbic acid, butylated hydroxytoluene (BHT), 2′,7′-dichlorofluorescein diacetate (DCFH-DA), dihydroethidium (DHE), dimethyl sulfoxide (DMSO), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferrozine, linoleic acid, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-teyrazolium bromide (MTT), β-nicotinamide adenine dinucleotide (β-NADH), and α-tocopherol were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Isovitexin, orientin and vitexin were purchased from Fluka Chemika-Biochemika Co. (Ronkonkoma, NY, USA). Potassium dihydrogen phosphate (KH_2PO_4) and pyrogallol were obtained from Merck Co. (Darmstadt, Germany). Ferric chloride, calcium carbonate, and Tween 20 were purchased form Kanto Chemical Co. (Tokyo, Japan). Ammonium thiocyanate and sodium phosphate were purchased form Shimakyu Co. (Osaka, Japan). Phenazine methosulfate (PMS) and nitro blue terazolium chloride monohydrate (NBT) were purchased form Acros Organics (Phillipsburg, NJ, USA). Dulbecco's Modified Eagle Medium (DMEM) was purchased from GIBCOTM Co. (NY, USA). Fetal bovine serum (FBS) was purchased from BenchMark™ Gemin Bio-Products (Woodland, CA). Isoorientin was obtained from Chromadex Co. (Daimler St Santa Ana, CA).

2.2. Plant materials and growth conditions

Buckwheat seed was immersed in water for 6 h and then put into a bag for germination for 12–15 h. The seed was sown in a plate with sandy soil (35 × 25 × 3 cm). The plate was put in a plant incubator (Hotech, 624HD LE-539, Tai-

pei, Taiwan) at 25 ± 2 °C with 12 h sunlight and sprayed with water at intervals of 4 h everyday. The water used to grow buckwheat was TEW with the concentrations of 100, 200, 300, 400 and 500 ppm, while de-ionized water (DIW) was used as the control. The pH values of TEW at the concentration of 100, 200, 300, 400 and 500 ppm were 3.54, 3.28, 3.12, 3.01 and 2.88, respectively. Saturated $CaCO_3$ solution was used to adjust the pH of TEW to 6.2–6.5. After 6–7 days, buckwheat sprouts (about 5–7 cm) were harvested and freeze-dried. Dried buckwheat sprouts were milled and stored at 4 °C until use.

2.3. Composition determination

The analysis of the protein, lipid, moisture, ash, and fiber contents of buckwheat sprouts was performed according to standard AOAC methods (AOAC, 1997).

2.4. Determination of trace elements

Freeze-dried buckwheat sprout powder (3 g) or TEW was mixed with 10 ml of 70% nitric acid, and digested in a closed digestion oven. After being liquefied, the sample volume was adjusted to 50 ml, and analyzed by an inductively coupled plasma mass spectrometry (ICP-MS, Agilent Technologies 7500C, Tokyo, Japan). The amount of sample components were calculated by the obtained values deducted from the blank values in accordance with their standard curves.

2.5. Preparation of the ethanolic extract from buckwheat sprout

Freeze-dried buckwheat sprout powder (10 g) was extracted twice with 100 ml ethanol at 80 °C for 3 h using a rotary vacuum evaporator (EYELA N-N series, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The ethanolic solution was filtrated with Whatman No. 1 paper, and then ethanol was removed in an evaporator at temperature lower than 40 °C. The residue was freeze-dried (EYELA FDU-540, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) and stored at −20 °C until use.

2.6. Determination of flavonoid compounds with HPLC analysis

Buckwheat sprout powder or the ethanolic extract was dissolved in methanol and filtered through a nylon filter (0.45 μm). The contents of isoorientin, vitexin, isovitexin and rutin in the mixtures were determined using high performance liquid chromatography (HPLC) (Hewlett Packard series 1100, Agilent Technologies, Inc., Santa Clara, CA, USA) with an internal standard, pyrogallol. The conditions for HPLC were: Zorbax 80A Extend C18 column (5 μm, 4.6×250 mm, Agilent technologies, Inc., Santa Clara, CA, USA); 5 μl injection; 1 ml/min flow rate; ultraviolet detection at 254 nm. The mobile phases were water

(Mill-Q, 18.2 MΩ) (mobile phase A) and methanol:acetic acid (95:5, v/v) (mobile phase B). The gradient conditions were mobile phase B 0%, 20%, 40%, 50%, 70% and 100% at 0, 10, 20, 30, 40 and 508 min, respectively.

2.7. Determination of DPPH radical scavenging activity

DPPH radical-scavenging activity was measured according to the method of Shimada, Fujikawa, Yahara, and Nakamura (1992) with slight modification. The ethanolic extract (1 ml) was mixed with 1 ml 1 mM DPPH (in methanol), then the mixture was shaken vigorously and left in the dark at room temperature for 30 min. The absorbance of the resultant solution was measured by Helios Alpha UV–vis Spectrophotometer (Thermo Fisher Scientific, Inc., MA, USA) at 517 nm. The DPPH radical scavenging activity was calculated as follows: scavenging activity $(\%) = [(A_0 - (A - A_b))/A_0] \times 100\%$, where A_0 was value of DPPH without sample; A was value of sample and DPPH; A_b was value of sample without DPPH. Ascorbic acid, α-tocopherol and BHT were used as positive controls.

2.8. Determination of ferrous ion chelating activity

Ferrous ion chelating activity of the sample solution was determined by the method of Dinis, Maderia, and Almeda (1994). The sample solution (2 ml) was mixed with 0.6 ml de-ionized water and 0.1 ml 1 mM $FeCl_2$, and then set at room temperature for 30 s. The reaction was initiated by the addition of 0.2 ml 2.5 mM ferrozine for 10 min, the absorbance of the mixture was measured using a Helios Alpha UV–vis Spectrophotometer (Thermo Fisher Scientific, Inc., MA, USA) at 562 nm. Chelating activity $(\%) = [1 - (\text{sample } A_{562\,nm}/\text{blank } A_{562\,nm})] \pm 100\%$.

2.9. Determination of superoxide anion scavenging activity

Superoxide anion scavenging activity of the ethanolic extract from buckwheat sprout was based on the method described by Roback and Gryglewski (1988) with slight modification. One milli liter of nitroblue tetrazolium solution (150 μM in 100 mM phosphate buffer, pH 7.4), 1 ml NADH solution (468 μM in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of the ethanolic extract from buckwheat sprout were mixed. The reaction was started by adding 100 μl of phenazine methosulphate solution (60 μM in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25 °C for 58 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula: % scavenging activity $= [(A_0 - A_1)/A_0] \times 100$; where A_0 was the absorbance of the control, and A_1 was the absorbance of the buckwheat sprout extract.

2.10. Determination of the inhibitory activity toward lipid peroxidation

The antioxidant activity was determined according to the method described by Misuda, Yasumoto, and Iwami (1966) with some modification. A 2.5 ml aliquot of 0.02 M linoleic acid emulsion (0.2804 g linoleic acid and 0.2804 g Tween 20 in 50 ml phosphate buffer, pH 7.0) was mixed with 0.5 ml sample solution and 2 ml of 0.2 M phosphate buffer (pH 7.0). After incubation at 50 °C in the dark for 72 h, a 0.1 ml aliquot of the reaction solution was mixed with 4.7 ml of ethanol (75%), 0.1 ml of ammonium thiocyanate (30%) and 0.1 ml of ferrous chloride (20 mM). After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm, and the inhibition percent of linoleic acid peroxidation was calculated as $(\%)$ inhibition $= [1 - (\text{absorbance of sample at } 500\,nm)/(\text{absorbance of control at } 500\,nm)] \times 100$. Ascorbic acid, α-tocopherol and BHT (0.1 mg/ml) were used as positive control.

2.11. Cell culture

Human hepatoma HepG2 cells were purchased from Food Industry Research and Development Institute, Hsinchu, Taiwan. The cells were grown in a humidified incubator (90% humidity) with 5% CO_2 at 37 °C. They were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% Biowhitaker fetal bovine serum (FBS). The culture medium was changed every other day and the cells were usually split 1:4 when they reached 80–90% confluence.

2.12. Determination of cell viability

The cells were plated in 96-well plates at an initial cell count of 1×10^4 per well and incubated at 37 °C, 5% CO_2. After treating with different concentrations (0.05–1 mg/ml) of 100 μl ethanolic extract from buckwheat sprout for 24–72 h, the cells were incubated with 2 mg/ml 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (100 μl) for 2.5 h. Then the intracellular formazan product was dissolved in dimethyl sulfoxide (DMSO) (100 μl) and quantified with an ELISA reader at 570 nm.

2.13. Determination of antioxidant enzymes activity

The cells were plated in 60 mm diameter plates at a concentration of 4×10^6 per plate and incubated at 37 °C, 5% CO_2. The cells were pretreated with 5 ml of 0.5 mg/ml ethanolic extract from buckwheat sprout for 6 h. Then the plate was washed with phosphate buffered saline (PBS) twice. The cells were collected and broken by an ultrasonic processor, and then the supernatant was collected by centrifugation at 10,000 rpm, 4 °C for 30 min. Superoxide dismutase (SOD) activity was determined by the commercial kit, RANSOD SD-125 assay kit (Randox Laboratories

Ltd., Ardmore, UK), while GPx activity was determined by RANSEL RS-504 assay kit (Randox Laboratories Ltd., Ardmore, UK).

2.14. Determination of intracellular reactive oxygen species (ROS) and superoxide anion

The intracellular ROS production was measured using a nonfluorescent compound 2′,7′-dichlorofluorescein diacetate (DCFH-DA) (LeBel, Ishiropoulos, & Bondy, 1992). When this compound enters viable cells, it can be deacetylated to form 2′,7′-dichlorofluorescin (DCFH) which can react quantitatively with ROC within the cell to produce 2′,7′-dichlorofluorescein (DCF), which is fluorescent. In flow cytometry, DCF can produce green fluorescent (535 nm) and provide an index of intracellular oxidation (Wang & Joseph, 1999). Superoxide anion can oxidize a membrane permeable substrate, dihydroethidium (DHE) to form a fluorescent compound, ethidium. In flow cytometry, ethidium can produce red fluorescence (605 nm) and provide an index of intracellular level of superoxide anion.

The cells were plated in 60 mm diameter plates at a concentration of 4×10^6 per plate and incubated at 37 °C, 5% CO_2. The cells were pretreated with 5 l of 0.5 mg/ml ethanolic extract from buckwheat sprout for 3, 6, 9 and 12 h. Then 2 ml of 10 μM DCFH-DA or DHE was added and further incubated for 30 min. Then the plate was washed with PBS twice. The cells were collected in a 15 ml centrifuge tube and then centrifuged at 1000 rpm for 5 min to remove the supernatant. And then 0.7 μl PBS was used to transfer the cells to a polypropylene conical tube. ROS and superoxide anion measurements were performed using a flow cytometer (fluorescence-activated cell sorter, FACSCalibur flow cytometer, Becton Dickinson, San Jose, CA) with the CellQuest software.

2.15. Statistical analysis

All data were triplicate and expressed as mean ± standard deviation. Analysis of variance was performed by ANOVA procedures. Duncan's multiple-range test was used to determine the difference of means, and $p < 0.05$ was considered to be statistically significant. Student's t-test was used to determine the difference between two means.

3. Results and discussion

3.1. Effects of TEW concentrations on the antioxidant activities of ethanolic extracts from buckwheat sprout

Fig. 1 shows the antioxidant activities of ethanolic extracts (1 mg/ml) from buckwheat sprout grown in DIW and TEW at 100, 200, 300, 400 and 500 ppm. It was found that buckwheat sprout grown in TEW at 300 ppm had the highest DPPH radical scavenging activity, ferrous ion chelating activity and inhibitory activity toward lipid peroxi-

dation. Further increase of the concentration of TEW could result in a decline in the antioxidative activities. However, the concentration of TEW showed no significant effect on the superoxide anion scavenging activity. Therefore, we concluded that TEW at 300 ppm yielded the highest antioxidant activity of buckwheat sprout. Thus, for the subsequent tests, we only selected 300 ppm TEW as the experimental factor using DIW as control. The amount of ethanolic extracts from buckwheat sprouts grown at DIW and 300 ppm TEW were not significantly different, 21.57 ± 0.44% and 23.61 ± 1.84%, respectively.

3.2. The trace element contents, composition and flavonoid contents of buckwheat sprout

TEW contains more than 80 trace elements. We determined the amounts of Cu, Zn, Se, Mn and Fe because they might relate to the antioxidant ability. Their contents in 300 ppm TEW and buckwheat sprout are shown in Table 1. All five trace elements were significantly different between TEW and DIW. In buckwheat sprout, the amounts of Cu, Zn and Fe were higher in TEW group than DIW group, but the amount of Se and Mn did not differ between TEW and DIW groups. The results indicated that increase of these trace elements in buckwheat sprout was due to the supplementation of TEW. Therefore, this implied that the possible harmful trace elements presented in TEW might also be absorbed by buckwheat. So the amount of Cr, As, Cd, Hg and Pb in TEW were also determined and the data are shown in Table 2. It was found that all the tested heavy metal contents did not differ between TEW and DIW with the exception of Cr content. Although the Cr content in TEW was 2.2 fold of that in DIW, the amount (2.67 μg/g) was not a problem in terms of safety considerations.

Buckwheat sprout grown at 300 ppm TEW had higher crude ash then that grown in DIW, but no significant differences were found in moisture, crude protein, crude fat and crude fiber contents (Table 3). This indicated that the supplement of TEW raised the level of mineral content in buckwheat sprout and increased the crude ash content.

Three different flavonoid compounds were found in buckwheat sprout, their contents followed the order: rutin (7.62 mg/g) > quercitrin (0.21 mg/g) > quercetin (0.16 mg/g) in wet weight basis (Fig. 2). All the flavonoid compounds did not differ between fresh buckwheat sprouts grown in TEW and DIW. Moreover, in the ethanolic extracts, all the flavonoid contents were similar in TEW and DIW groups (data not shown).

3.3. Antioxidant activities of ethanolic extracts from buckwheat sprout

Antioxidant activities of the ethanolic extracts from buckwheat sprout grown in TEW (300 ppm) and DIW were determined at six concentrations, 0.31, 0.63, 1.25, 2.5, 5 and 10 mg/ml. There was significant difference ($p < 0.01$) in the DPPH radical scavenging activity between
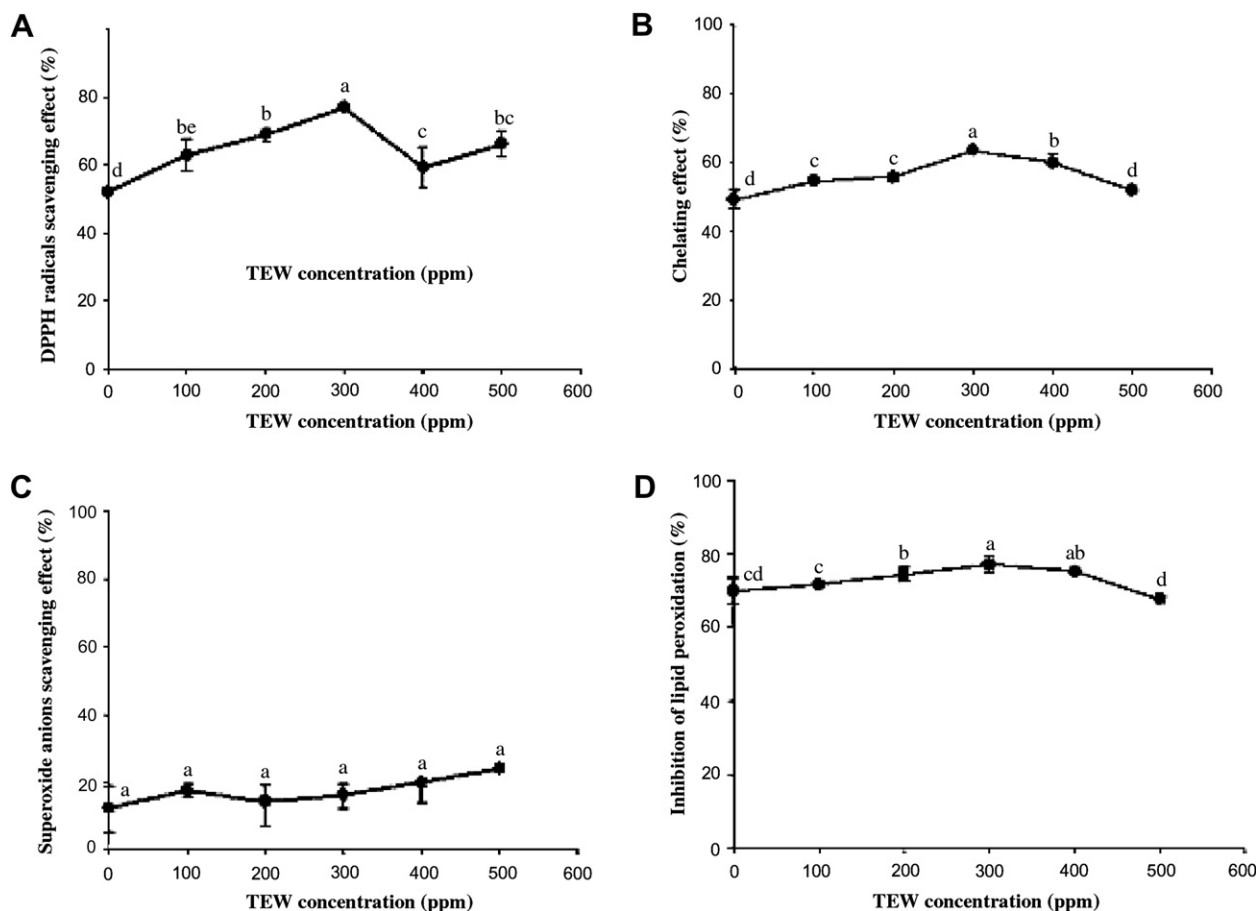
Fig. 1. Effects of the concentrations of trace element water on the antioxidative activities of the ethanolic extracts from buckwheat sprout: (A) DPPH radical scavenging activity, (B) ferrous ion chelating ability, (C) superoxide anion scavenging activity and (D) inhibition toward lipid peroxidation. ([a–d]The values followed by different superscripts were significantly different at significant level of 0.05.)

Table 1
The contents of trace elements in trace element water (at 300 ppm), de-ionized water, and buckwheat sprout

µg/g	Cu	Zn	Se	Mn	Fe
Water					
DIW	0.30 ± 0.003^b	8.76 ± 0.002^b	0.13 ± 0.04^b	0.15 ± 0.001^b	1.01 ± 0.01^b
TEW	2.42 ± 0.002^a	37.24 ± 0.004^a	0.28 ± 0.03^a	49.77 ± 0.02^a	2391 ± 0.00^a
Buckwheat sprout					
DIW group	0.61 ± 0.04^b	3.69 ± 0.03^b	0.004 ± 0.001^a	5.41 ± 0.01^a	14.14 ± 6.57^b
TEW group	0.74 ± 0.01^a	4.86 ± 0.09^a	0.007 ± 0.002^a	5.34 ± 0.15^a	22.38 ± 0.84^a

The contents are presented on the dry weight basis.
The values are mean $\pm$ standard deviation ($n = 3$).
[a,b] Values in the same column followed by different superscripts are significantly different ($p < 0.05$).

Table 2
Heavy metal contents in buckwheat sprouts grown in trace element water (at 300 ppm) and de-ionized water

| Unit (µg/g)	Cr	As	Cd	Hg	Pb
DIW group	1.22 ± 0.06^b	0.0022 ± 0.0004^b	0.006 ± 0.000^a	0.002 ± 0.001^a	0.0164 ± 0.0039^a
TEW group	2.67 ± 0.05^a	0.0044 ± 0.0004^a	0.006 ± 0.000^a	0.0009 ± 0.0004^a	0.0100 ± 0.0007^b

The contents are presented on the dry weight basis.
The values are mean $\pm$ standard deviation ($n = 3$).
[a,b] Values in the same column followed by different superscripts are significantly different ($p < 0.05$).

Table 3
The compositions of buckwheat sprouts grown in trace element water (at 300 ppm) and de-ionized water

%	Moisture	Crude protein	Crude fat	Crude fiber	Crude ash
DIW	92.34 ± 0.41^a	0.14 ± 0.01^a	0.30 ± 0.03^a	0.73 ± 0.10^a	0.48 ± 0.01^b
TEW	91.69 ± 1.08^a	0.15 ± 0.01^a	0.31 ± 0.01^a	0.69 ± 0.05^a	0.58 ± 0.002^a

The contents are presented on the wet weight basis.
The values are mean $\pm$ standard deviation ($n = 3$).
[a,b] Values in the same column followed by different superscripts are significantly different ($p < 0.05$).
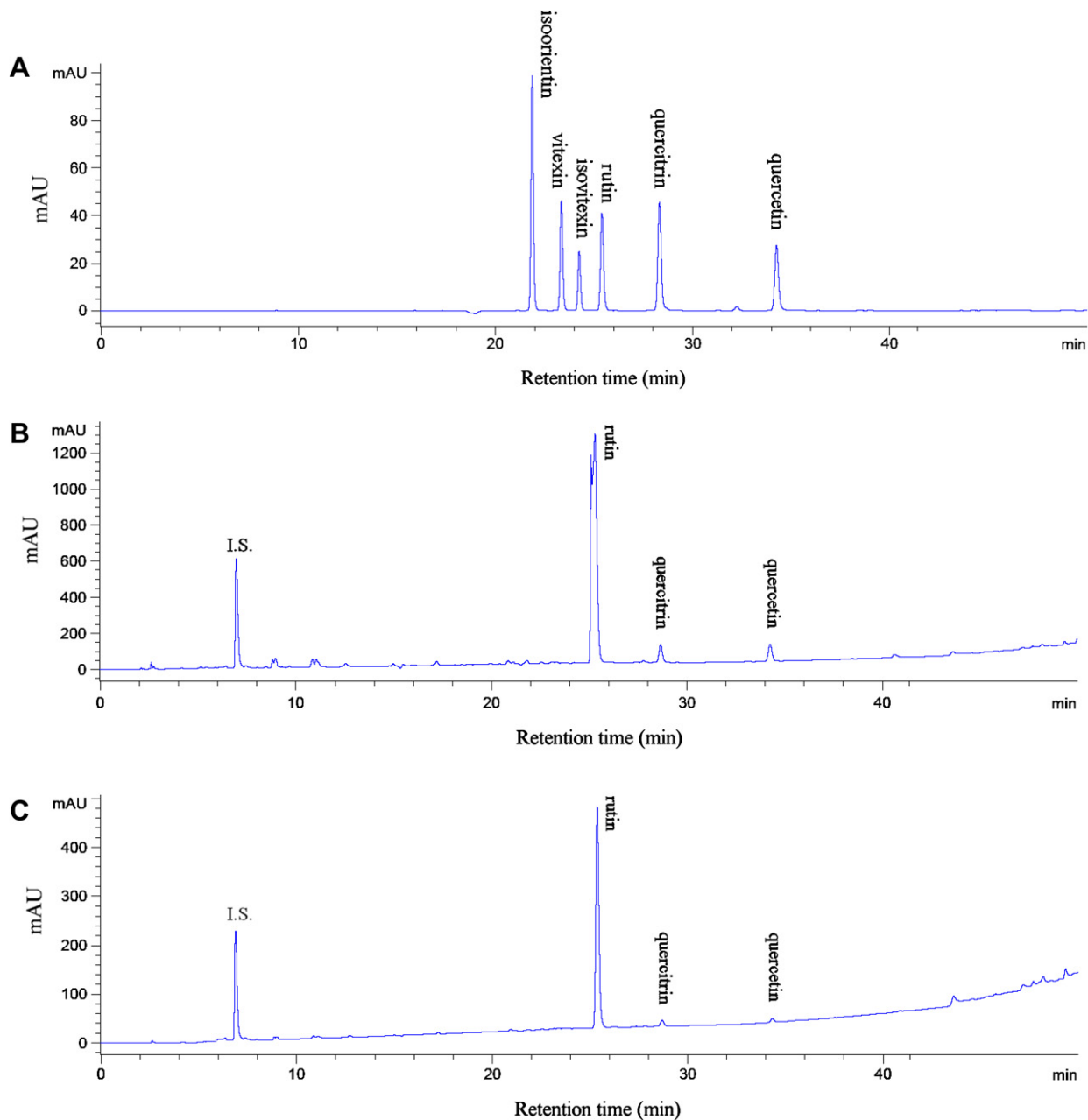
Fig. 2. Flavonoid contents in the ethanolic extract from buckwheat sprout determined by HPLC: (A) standard material, (B) DIW group and (C) TEW group.

the ethanolic extracts from buckwheat sprouts grown in TEW and DIW at the concentrations of 0.63 mg/ml or higher (Fig. 3A). When compared to ascorbic acid, α-tocopherol and BHT, the DPPH radical scavenging

activity in the extract from buckwheat sprout was lower than that of all three antioxidants. Our data also showed the extract from TEW group showed significantly higher ferrous ion chelating activity than the extract from DIW
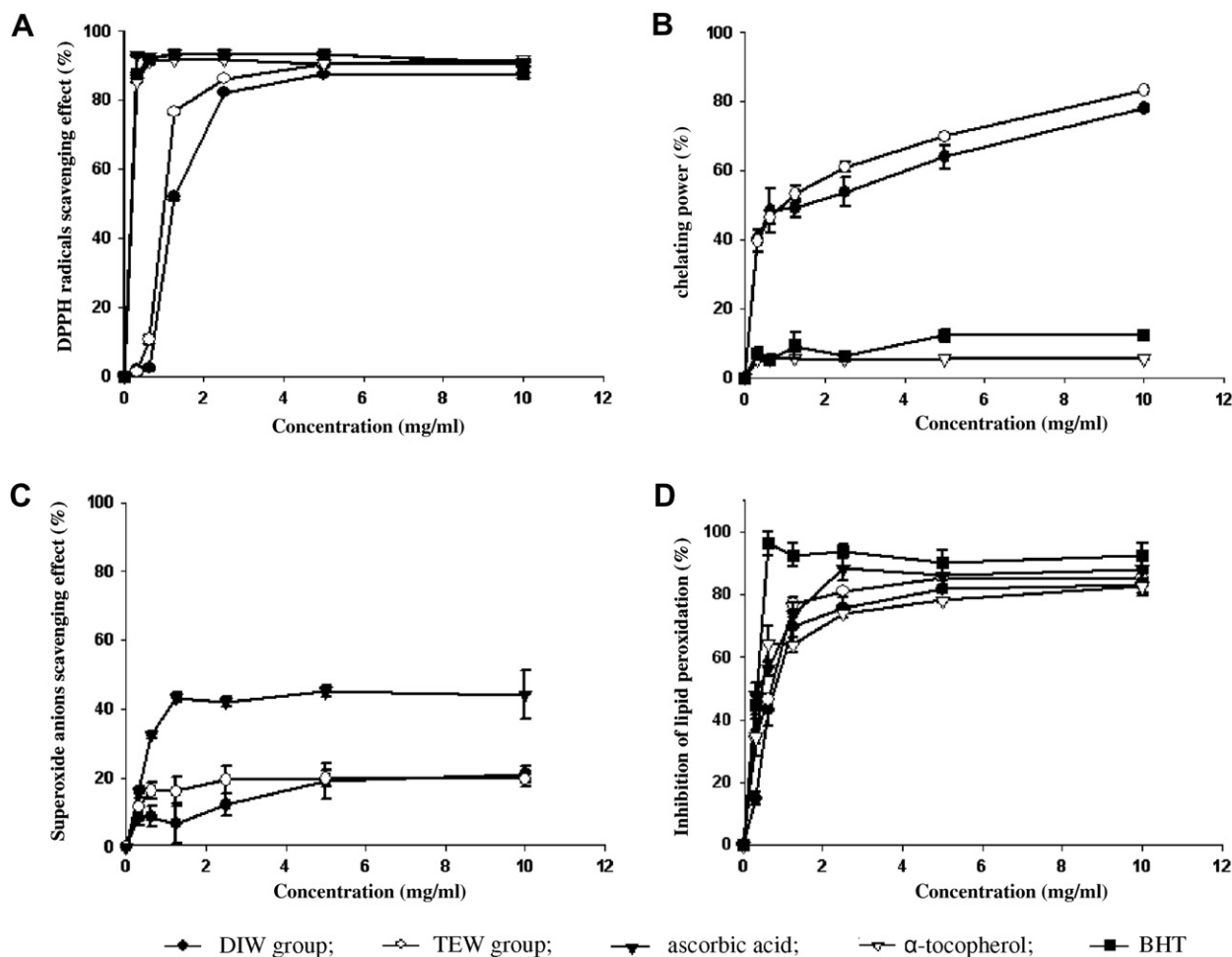
Fig. 3. Antioxidative activities of the ethanolic extract of buckwheat sprout grown in DIW and TEW: (A) DPPH radical scavenging activity, (B) ferrous ion chelating ability, (C) superoxide anion scavenging activity and (D) inhibition toward lipid peroxidation.

group ($p < 0.01$) at concentrations of 1.25 mg/ml or higher (Fig. 3B). Since α-tocopherol and BHT do not have metal ion chelating functional groups, they showed poor ferrous ion cheating activity. Our data also showed that ascorbic acid did not have the ability to bond ferrous ion. It has been pointed out that metal ions from $FeCl_3$, $CuCl_2$ or $FeCl_2$ can promote the peroxidation of ascorbic acid and decrease its ferrous ion chelating activity (Satoh & Sakagami, 1997). Both the TEW and DIW groups showed low superoxide anion scavenging activities, but TEW groups had slightly higher scavenging activity than DIW groups (Fig. 3C). Ascorbic acid had high superoxide anion scavenging activity, but α-tocopherol and BHT did not show inhibitory activity toward superoxide anions. In fact, Kim, Han, Moon, and Rhee (1995) even reported that α-tocopherol and BHT promoted the production of superoxide anions. The ability to inhibit the lipid peroxidation was higher in TEW group than in DIE group ($p < 0.01$) in the concentration range of 0.31–5 mg/ml. It was found that the inhibitory activity of the ethanolic extract from buckwheat sprout was similar to those of α-tocopherol and ascorbic acid, but lower than that of BHT (Fig. 3D).

In general, our data indicated that the ethanolic extract from buckwheat sprout showed high reducing power, DPPH radical scavenging activity, ferrous ion chelating activity and inhibitory activity toward lipid peroxidation, but poor superoxide anion scavenging activity. And buckwheat sprout grown in TEW group had higher DPPH radical scavenging activity, reducing power, ferrous ion chelating activity, superoxide anion scavenging activity and inhibitory activity toward lipid peroxidation than the sprout grown in DIW group. Since the levels of rutin and other tested flavonoid compounds did not differ between buckwheat sprout grown in TEW and DIW, but the levels of tested trace elements did increase in the TEW group, the higher antioxidant activity in the TEW group might due to the trace elements in TEW.

3.4. Cell viability

Effects of the ethanolic extracts from buckwheat sprouts grown in TEW and DIW on the viability of human Hep G2 cell were tested at five different concentrations, 0.05, 0.1, 0.2, 0.5 and 1 mg/ml. At low concentrations, 0.05 and
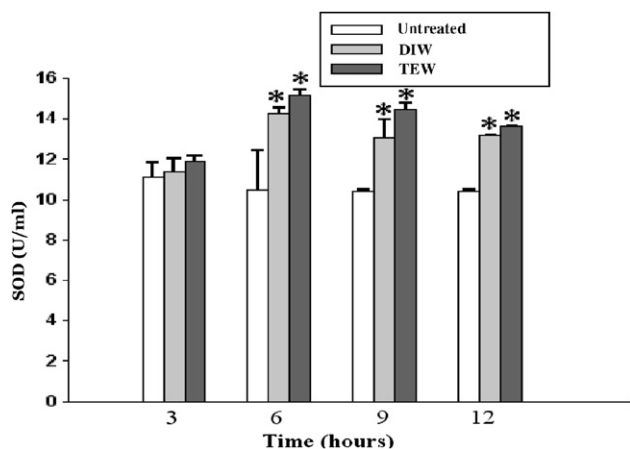
Fig. 4. Effect of the ethanolic extract from buckwheat sprout on the superoxide dismutase (SOD) activity in human Hep G2 cells. ([*]: The value is significant difference to the untreated value at each sampling time.)

0.1 mg/ml, both the ethanolic extracts in TEW and DIW groups showed no cyto-toxicity toward human Hep G2 cells. At high concentrations, 0.5 mg and 1 mg/ml, significant toxicity was observed in both the extracts in TEW and DIW. We selected the medium concentration, 0.2 mg/ml, for the subsequent tests for evaluating the anti-oxidant status in human Hep G2 cells because the cell viability was maintained higher than 80% up to 72 h. It was also noted that effect of the extract from buckwheat sprout did not differ between TEW and DIW groups at all tested concentrations.

3.5. Intracellular activities of SOD and GPx

Human Hep G2 cells were pretreated with 0.2 mg/ml of the ethanolic extracts in TEW and DIW groups for 3, 6, 9 and 12 h. The effect of the extracts on the intracellular SOD activity was determined and the data are shown in Fig. 4. It was found that the pretreatment of the extracts significantly increased the SOD activity ($p < 0.01$). When pretreated with the extracts for 6, 9 and 12 h, the SOD activity was higher in TEW group than that in DIW group ($p < 0.001$). This might be because the extract in TEW group had higher trace elements ($p < 0.01$), such as Cu and Zn, that could promote the SOD activity. As shown in Fig. 5, the pretreatment of the extracts also increased the GPx activity in human Hep G2 cells at 6 and 9 h ($p < 0.01$); however, no significant difference was found between the extracts in TEW and DIW groups. It has been shown that Se can influence GPx activity (Brigelius, 1999; Tinggi, 2003). It was suspected that the amount of Se did not differ in buckwheat sprout grown in TEW and DIW, therefore, no difference was found in the GPx activity.

3.6. Intracellular ROS and superoxide anion

The fluorescence intensity of DCF was used as the index to represent the amount of intracellular ROS level in human Hep G2 cells. As shown in Fig. 6, the extracts from
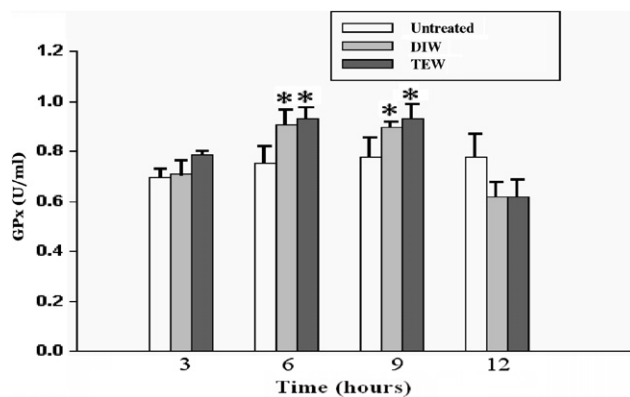

Fig. 5. Effect of the ethanolic extract from buckwheat sprout on the glutathione peroxidase (GPx) activity in human Hep G2 cells. ([*]: The value is significant difference to the untreated value at each sampling time.)
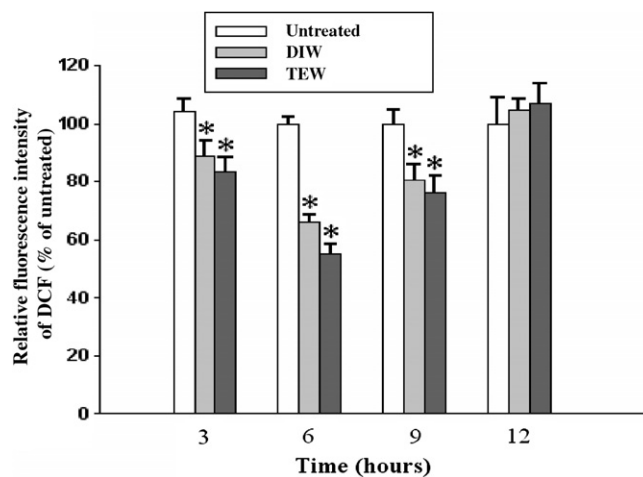

Fig. 6. Effect of the ethanolic extract from buckwheat sprout on the content of reactive oxygen species (ROS) in human Hep G2 cells. ([*]: The value is significant difference to the untreated value at each sampling time.)
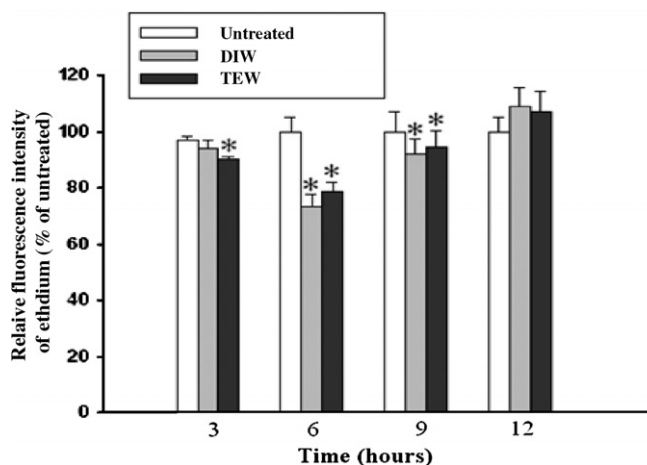

Fig. 7. Effect of the ethanolic extract from buckwheat sprout on the content of superoxide anion in human Hep G2 cells. ([*]: The value is significant difference to the untreated value at each sampling time.)

both TEW and DIW groups reduced the production of ROS when the cells were pretreated with the extract for 3, 6 and 9 h. Moreover, at 6 h the extract from TEW group resulted in significantly lower ROS level than the extract from DIW group. This finding agreed with higher SOD activities found in TEW group at 6 h because in TEW group SOD might convert ROS to hydrogen peroxide more effectively. Our data indicated that the supplement of TEW to buckwheat increased the activity of SOD, thus reduced the levels of ROS in human Hep G2 cells. The extracts from buckwheat also significantly decreased the level of superoxide anions in the cells (Fig. 7). However, the production of intracellular superoxide anions did not vary between TEW and DIW groups.

4. Conclusions

The application of trace element water to enhance the antioxidant status of buckwheat sprout appears to be promising. Although it was still not clear what the key trace elements were responsible for the increase of the antioxidant activities, we have demonstrated that the beneficial effect of trace elements on the antioxidant activity could be accomplished with the supplementation of trace element water.

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