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ORIGINAL ARTICLE

Chelating Effect of Leek (*Allium tuberosum* Rottler ex Sprengel) Containing Chlorophyll on Cd, Pb, and As

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Abstract Effect of leek (Chinese chive) on bioaccessibility of arsenic (As), cadmium (Cd), and lead (Pb) was determined in comparison with sodium copper chlorophyllin (SCC) using *in vitro* digestion model. Leek (0, 6, 12, 60, and 120 mg) and SCC (0, 1, 5, 10, and 50 mg) were digested with equal concentration (10 ppm) of As, Cd, and Pb. Concentration of each heavy metal in aqueous phase following *in vitro* digestion was measured using an inductively coupled plasma optical emission spectrometer. Changes in absorbance spectra of chlorophyll extracted from leek and SCC by heavy metals were measured at 0, 1, 2, and 3 h after mixing. Results showed that the concentration of each heavy metal in aqueous phase decreased with increasing amounts of leek and SCC. At the highest level of leek (120 mg), the concentrations of As, Cd, and Pb decreased to 93.9, 87.1, and 58.2%, respectively. Absorbance of chlorophyll drastically decreased after 1 h of mixing with each As, Cd, and Pb, and no difference in the absorbance was observed after 2 and 3 h. This result indicates that mixing chlorophyll extracted from leek with each heavy metal for 1 h was sufficient for chelating As, Cd, and Pb ions. On the other hand, SCC and each heavy metal showed a decreasing pattern of absorbance without any significant difference for 3 h, indicating that chlorophyll from leek was more effective than SCC, a commercial grade chlorophyll derivative, in chelating As, Cd, and Pb. Results showed leek reduces heavy metals in humans.

Keywords bioaccessibility · chelating · chlorophyll · heavy metal · leek

Introduction

Food safety related to heavy metal contamination is becoming a significant problem worldwide, because the presence of heavy metals found in foodstuffs are toxic and mutagenic even at very low concentrations (Bahemuka and Mobofu, 1999; Gupta et al., 2010; Cui et al., 2011). Chronic exposure to the low level of heavy metals causes negative effects on human beings such as disease, malfunction, and malformation of organs, because effective mechanism to remove heavy metals is not yet available (Radwan and Salama, 2006; Gupta et al., 2010).

For arsenic (As), the prolonged exposure to As compounds, which easily dissolve in water, results in fatal damages including anemia, leucopenia, and some internal cancers involved in skin, bladder, kidney, and lung (Wang and Mulligan, 2006; Sirot et al., 2009). As to the chronic exposure to cadmium (Cd), nephrotoxicity was caused by cadmium-contaminated rice, and debilitating osteoporosis was affected by the high level of environmental cadmium (Waalkes 2003; Gupta et al., 2010; Kazi et al., 2010). Pb also causes damages to the brain and peripheral nerves (Abdel-Aal et al., 2011), and Pb-poisoned children showed such symptoms as vomiting, anorexia, and abdominal cramps (Lanphear et al., 2003). These heavy metals may be taken in directly by the crop as well as through shifting to the food chain (Devkota and Schmidt, 2000). Therefore a synthetic chelator (e.g. D-penicillamine) to reduce the lead level in blood was utilized, even though it had fatal side effects including platelet depression, leucopenia, and adverse reactions (Golalipour et al., 2007). Accordingly, study on the alternative food factors which effectively inhibit bioaccessibility of As, Cd, and Pb was performed.

Chinese chive (*Allium tuberosum* Rottler ex Sprengel) belongs to the *Allium* genus, which is abundant in steroidal saponins, alkaloids, and sulfur-containing compounds, and can grow to more than 45 cm (Hu et al., 2006; Kawagishi e al., 2009). It is a perennial herb and harvested in East Asia including Korea, Japan, and China and South Eastern Asia including Thailand, Indonesia,

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Vietnam, and Philippines (Imahori et al., 2004; Yabuki et al., 2010). It is well used vegetable for foods, herbal remedies, and spices worldwide (Sang et al., 2003; Hu et al., 2006; Kawagishi et al., 2009). The leaves of Chinese chive have been employed for treating abdominal pain, diarrhea, hematemesis, snakebite, and asthma as reported in a previous study (Sang et al., 2003). Chinese chive contains chlorophyll, β-carotene, and vitamin C, which show an antioxidative effect involved in scavenging free radicals (Kwak and Kim, 2009).

Chlorophyll found in leek is the most common pigment representing green and used for therapy (Ferruzzi and Blakeslee, 2007). Chlorophyll is known to associate with heavy metals, for instance, it was unveiled that the level of chlorophyll decreased as heavy metals entered into the photobiont cells (Chettri et al., 1998). Furthermore, Choi et al. (2003) reported that sodium copper chlorophyllin (SCC) had the capacity to inhibit mercury absorption after going through *in vitro* digestion in a human intestinal cell model. Hence this result implied chlorophyllin, a synthetic water-soluble condition of chlorophyll, could be effective on inhibiting absorption of heavy metals (Hwang and Shim, 2008).

There have been limited studies on the effects of safe food factors on chelating heavy metals, compared with the synthetic chelating substance including D-penicillamine (Kamat et al., 2000; Lee et al., 2009; Park et al., 2009). As shown above, studies on Chinese chive containing chlorophyll could unveil more data on Chinese chive in searching for food factors possessing the ability to inhibit the intake of heavy metals. Thus, the aim of the present study was to determine the effects of Chinese chive containing chlorophyll on heavy metals including As, Cd, and Pb.

Materials and Methods

Sample preparation. Chinese chive (*A. tuberosum* Rottler ex Sprengel) was obtained from a local field in Gwangyang-Si, Jeollanam-Do, Korea in October, 2010. Samples of selected Chinese chives without signs of wilting were washed with distilled water and immediately lypophilized. They were ground in a dark room and kept at –20ºC until further analysis.

Materials. SCC, D-penicilalamine, heavy metals including As, Cd, Pb, and digestive enzymes including amylase, pepsin, lipase, pancreatin, and bile acid were purchased from Sigma-Aldrich (St. Louis, Mo).

***In vitro* digestive system.** *In vitro* digestive model system was used as described by Shim et al. (2010) with slight modifications. Aliquots (0, 6, 12, 60, and 120 mg) of freeze-dried Chinese chive were dissolved in 20 mM phosphate buffer. Each heavy metal (As, Cd, and Pb) (10 ppm) was added to the samples. To make the salivary phase, amylase (0.5 mg/mL in 20 mM phosphate buffer) was added, and its initial pH was adjusted to pH 6.9 with the addition of 20 mM phosphate buffer. After treating samples with nitrogen gas, they were incubated in a shaking bath (37ºC, 250 rpm) for 5 min. To initiate the gastric phase, pepsin (3 mg/mL in 100 mM HCl) was added and its pH was adjusted to pH 2.0 by adding 1 M HCl. Samples were incubated in a shaking bath (37ºC, 250 rpm) for 1 h. For the small intestinal phase, its pH was adjusted to pH 5.3 with 1 M sodium bicarbonate solution. Bile acid (2.4 mg/mL in 20 mM phosphate buffer) and small intestinal enzyme solution including lipase (0.2 mg/mL in 20 mM phosphate buffer) and pancreatin (0.4 mg/mL in 20 mM phosphate buffer) were added to samples, and its pH was adjusted to pH 7.0 by addition of 1 M NaOH. After finishing three stages of *in vitro* digestive model system including salivary, gastric, and small intestinal phases, all samples were added with 20 mM phosphate buffer to achieve equal volumes, treated with nitrogen gas, and were incubated in a shaking bath (37ºC, 250 rpm) for 2 h. Each supernatant of Chinese chive was centrifuged at 4ºC and 3000 rpm for 30 min to isolate the supernatant from the particulate residue. The supernatants were stored at –80ºC until further analysis. Negative control (As, Cd, and Pb only), positive control (D-penicilalamine only), and SCC were treated with same procedures described above.

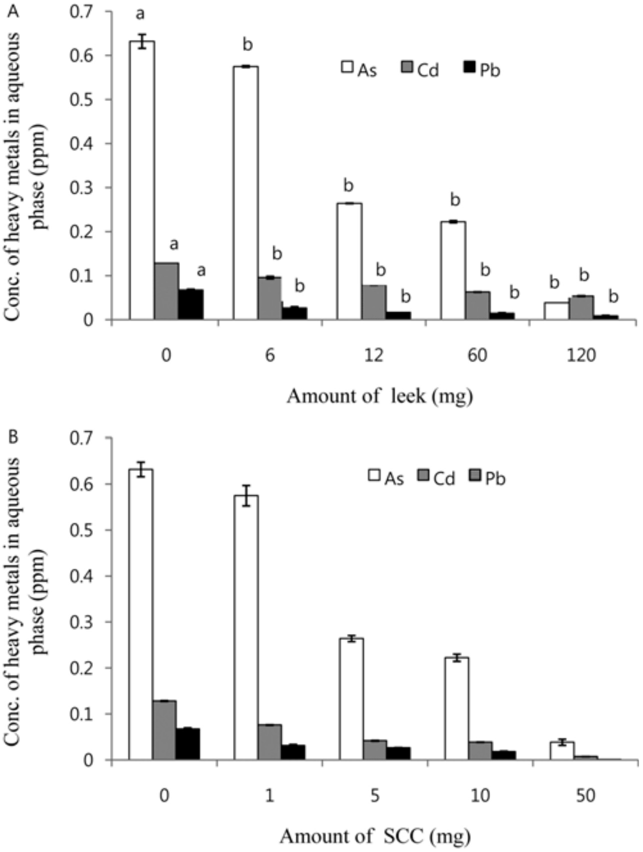

Fig. 1 Heavy metal concentration in aqueous phase following *in vitro*-digestion of each heavy metal (As, Cd, Pb; each at 10 ppm) in the presence of increasing amounts of leek (A) and SCC (B). Aqueous phase includes liquid that was decanted from pellet. Values represent means ± SE. Different letters indicate significant difference from negative control at p <0.05.

Determination of As, Cd, and Pb. To investigate the effects of Chinese chive, SCC, and D-penicillamine on inhibiting heavy metals, equal concentration of As, Cd, and Pb was added to increasing amounts of Chinese chive (0, 6, 12, 60, 120 mg), SCC (0, 1, 5, 10, 50 mg), and D-penicillamine (0.05, 0.1, 0.5, 1 mg), and then heavy metal concentrations in the samples were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES Vista PRO, Varian, Palo Alto, CA) coupled with a cross flow nebulizer and a spray chamber.

Extraction of chlorophyll from Chinese chive. Aliquot (1 g) of Chinese chive was dissolved in 85% acetone (100 mL) overnight. After filtering through a glass filter, the extract was treated with sodium sulfate and vacuum-evaporated. Chlorophyll (0.5607 g) was obtained from Chinese chive.

Detection of chlorophyll, heavy metals, and SCC. Chlorophyll and SCC extracted in sodium acetate-acetic acid buffer were reacted with As, Cd, and Pb at the ratio of 1:1:1 (M:M:M), respectively. The absorbances of the reacted samples were detected after 0, 1, 2, and 3 h at wavelengths between 340 and 700 nm at 20 nm intervals using VersaMax™ Microplate Reader (Cape Cod, MA).

Statistical analysis. All measurements were triplicated, and data were expressed as means ± SEM. Statistical analyses were performed by using analysis of variance and Turkey's post-hoc test (SAS, Cary, NC) at a significance level of $p < 0.05$.

Results and Discussion

In order to determine the effect of leek on reducing bioaccessibility of heavy metals, the contents of heavy metals in aqueous phase after *in vitro* digestion with various amounts (0, 6, 12, 60, and 120 mg) of leek were measured (Fig. 1A). The contents of As, Cd, and Pb decreased with increasing amounts of leek, and at the highest amount of leek (120 mg), were reduced to 93.9, 87.1, and 58.2%, respectively. At the lowest level of leek (6 mg), the inhibition of bioaccessibility for Pb was the most effective, which was 2.5 times higher than those for As and Cd, possibly due to the difference in ion charge, i.e.; As has one more ion charge in contrast to Cd and Pb. Therefore, chelating effect of leek on heavy metals is likely to be affected by number of ion charges.

The inhibitory effect of SCC on the bioaccessibility of heavy metals was also determined (Fig. 1B). The levels of heavy metals were reduced in proportion to increasing amounts (0, 1, 5, 10, and 50 mg) of SCC, which showed a pattern similar to the result from a leek study (Choi et al., 2003). Comparable result was found in a previous study using *in vitro* digestion model that SCC significantly decreased mercury bioaccessibility (Choi et al., 2003). However, *in vivo* study to confirm chelating effect of leek extracts as well as SCC on heavy metals has to be further investigated.

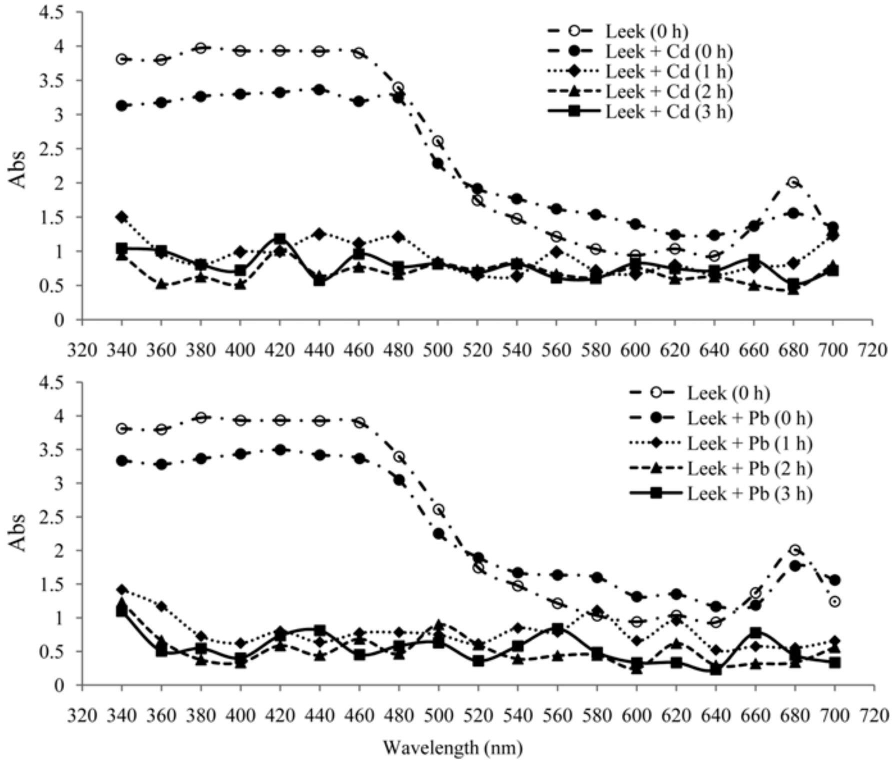

Fig. 2 Absorption spectra of chlorophyll extract from leek in presence of heavy metals (As, Cd, Pb) at room temperature at 0, 1, 2, and 3 h.

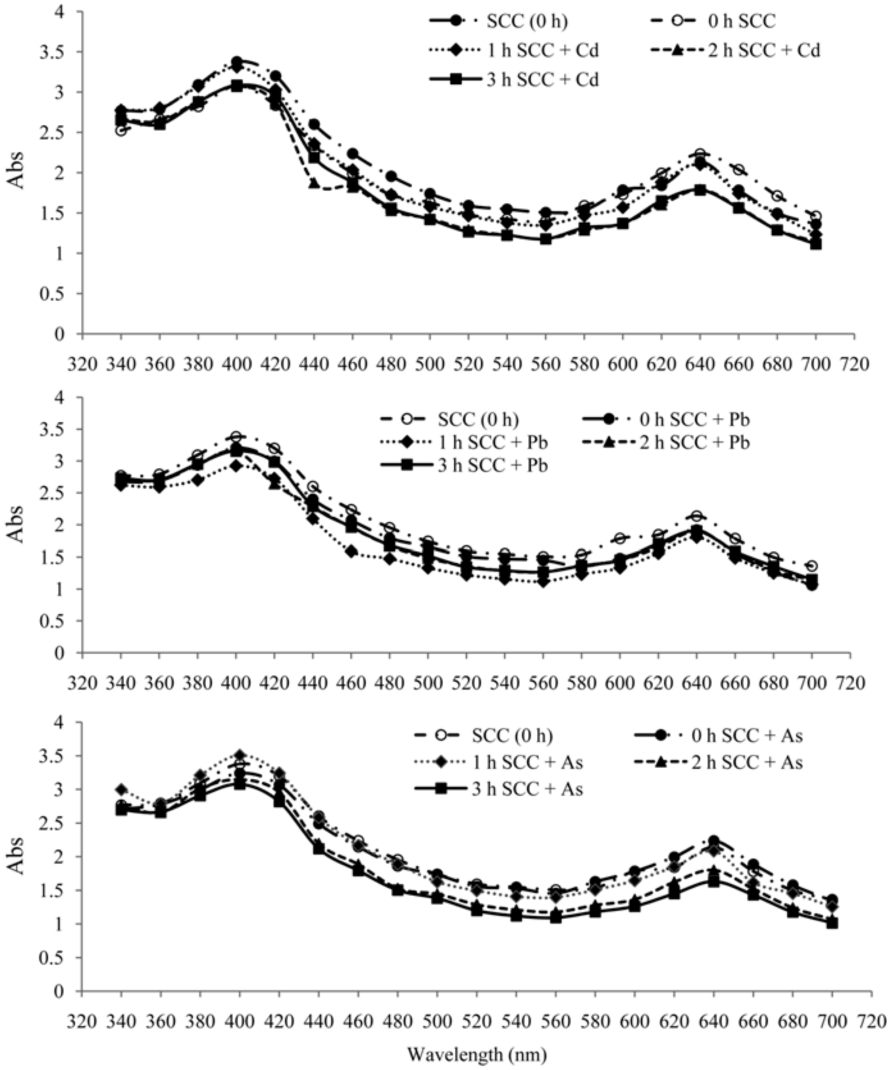

Fig. 3 Absorption spectra of SCC in presence of heavy metals (As, Cd, Pb) at room temperature at 0, 1, 2, and 3 h.

The present study also determined the chelating effect of leek on heavy metal ions by measuring absorbance spectra of a mixture of chlorophyll from leek and each As, Cd, and Pb (Fig. 2). The absorbance of chlorophyll drastically decreased after 1 h of mixing with each heavy metal with no difference in the absorbance after 2 and 3 h. This result indicates that mixing chlorophyll extracted from leek with each heavy metal for 1 h is sufficient for chelating As, Cd, and Pb, whereas a mixture of SCC and each heavy metal showed a decreasing pattern of absorbance with increasing reaction time of 0, 1, 2, and 3 h with no significant difference.

Results of the present study showed that chelation of chlorophyll or SCC with each heavy metal results in the bathochromic change of its absorbance. It has been known that chelation of flavonoids such as chlorophyll with specific metal ions is due to a high reducing power of flavonoids (Cho, 2003) that chelation of chlorophyll or SCC with heavy metal ions in human body can improve resistance against oxidative stress in a cell by cellular exposure to heavy metals.

In conclusion, results of the present study suggest that chlorophyll from leek can be a good chelator for reducing the level of heavy metals in humans, more effective than SCC, a commercial grade chlorophyll derivative, in terms of chelating power of heavy metals, As, Cd, and Pb. Further study is required for determining the effect of leek on intracellular uptake of these heavy metals.

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