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Characterisation, extraction efficiency, stability and antioxidant activity of phytonutrients in *Angelica keiskei*

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ABSTRACT

Phytonutrients in *Angelica keiskei,* a dark green leafy vegetable, were characterised and their extraction efficiency by different compositions of water/ethanol as well as stability at different temperatures was determined. A range in the content of lutein (205–265 mg/kg dry wt), *trans*-β-carotene (103–130 mg/kg dry wt), and total phenols (8.6–9.7 g/kg) was observed amongst *Angelica keiskei* grown in three different conditions. LC-ESI-MS/MS analysis identified chlorogenic acid, chalcones and the glucosides of luteolin and quercetin as the major phenolic compounds in *Angelica keiskei*. Only 46% of lutein was extracted by 70% of ethanol and no carotenoid was detected in 40% ethanol and 100% water extracts of *Angelica keiskei*. Major phytonutrients in *Angelica keiskei* were stable at −80 °C up to 12 months whilst β-carotene was significantly degraded at room temperature and 4 °C within 2 months and lutein at room temperature within 12 months.

The current study indicates that phytonutrients rich in *Angelica keiskei* can only be extracted partially using ethanol/water and substantial loss of certain phytonutrients such as β-carotene can occur during storage at either 4 °C or room temperature.

1. Introduction

Angel's plant (*Angelica keiskei*) is a large perennial plant belonging to the Umbelliferae family, grown originally in Japan. It is a dark green leafy vegetable that has been recognised as a medicinally important herb and is cultivated throughout Asia. Putative beneficial effects of *Angelica keiskei* reported in animal models include suppression of gastric acid secretion (Murakami et al., 1990), anti-tumourigenesis (Okuyama et al., 1991), anti-thrombosis (Fujita et al., 1992), antihypertension (Shimizu et al., 1999), suppression of histamine secretion, and vasodilation (Matsuura, Kimura, Nakata, Baba, & Okuda, 2001).

Extraction of phytonutrients in plant foods is a very critical step to condense the components in these foods, which can be of benefit as nutrient supplements, as well as to study the beneficial effects of these functional foods. In our previous studies, methanol and tetrahydrofuran (THF) have been used to successfully extract and quantify lipophilic phytonutrients in plant foods (Cho et al., 2007). As these solvents cannot be used for humans, ethanol can be the most suitable solvent for effective extraction of lipophilic phytonutrients in plant foods. In addition, a mixture of alcohol and water would improve the solubility of some phenolic compounds, such as ellagic acid, which is slightly soluble in water (Rangkadilok, Worasuttayangkurn, Bennett, & Satayavivad, 2005). However, the extraction efficacy of different compositions of alcohol and water has been seldom reported.

It has been reported that some antioxidants in foods are susceptible to degradation during storage (Aaby, Wrolstad, Ekeberg, & Skrede, 2007; Zafrilla, Ferreres, & Tomas-Barberan, 2001). Therefore, practical consideration must be given to the stability of these phytonutrients both in research studies and in monitoring consumer use.

The aims of the current study were to identify and characterise phytonutrients in *Angelica keiskei*, and to determine their extraction efficiency by different compositions of water/ethanol and stability at different temperatures for up to 12 months.

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2. Materials and methods

2.1. Chemicals and materials

All-*trans*-β-carotene, 9-*cis*-β-carotene, α-carotene, cryptoxanthin, lutein, zeaxanthin, lycopene, α-tocopherol, γ-tocopherol, methyl *tert*-butyl ether (MTBE), sodium chloride, sodium phosphate monobasic, sodium phosphate dibasic, sodium carbonate, Folin–Ciocalteu phenol reagent, potassium phosphate dibasic, Trolox and sodium salt fluorescein were obtained from Sigma Co. (St Louis, MO). Tetrahydrofuran (THF), water and methanol were purchased from JT Baker (Phillipsburg, NJ).

2.2. Sample preparation

Angelica keiskei was harvested from its major planted areas in Korea. Sample processing was conducted under dim light within 2 days of collection. After thorough cleaning using tap water, the *Angelica keiskei* samples were frozen for 24 h followed by drying for 35–36 h at 45–48 °C (1.5 tonne capacity, Dongil Cold Storage & Foods Co., Ltd., Busan, Korea). The dried *Angelica keiskei* was milled to powder using a 100-mesh sieve. The water content of fresh *Angelica keiskei* was 94.3% before processing and 1.9% after processing. Aliquots of 100 g each of sample were vacuum packed and immediately shipped to the Jean Mayer USDA-Human Nutrition Research Center on Aging at Tufts University within 2 months of sample collection. Samples were analysed for water-soluble and fat-soluble phytonutrients for baseline values upon arrival. In the meantime, aliquots of 100 mg each were placed in brown glass vials, nitrogen flushed, and stored at room temperature, 4 °C, and −80 °C for analysis of phytonutrient stability.

2.3. Phytonutrient extraction using 100% water, 40% ethanol, 70% ethanol

Different combinations of water and ethanol were used to extract phytonutrients in *Angelica keiskei* for considering future human use. Sample preparation was as follows. One kilogram of dried *Angelica keiskei* powder sample was extracted twice with 10 l each of distilled H_2O, 40% ethanol or 70% ethanol by stirring at 150 rpm for 48 h (EYELA NZ-1000, Tokyo Rikakikai Co. Ltd., Japan) at 4 °C for water extraction and room temperature for 40% and 70% ethanol extractions. Extracts were filtered and vacuum condensed at 450 mmHg at 45 °C (EYELA N-N, Tokyo Rikakikai Co. Ltd.), followed by lyophilisation (PVTFD10 R, ILshin Lab Co. Ltd., Seoul, Korea). Aliquots of 100 g each of sample were vacuum packed and immediately shipped to the Jean Mayer USDA-Human Nutrition Research Center on Aging at Tufts University. Samples were analysed upon arrival for water-soluble and fat-soluble phytonutrients for baseline values.

2.4. Analysis

2.4.1. Determination of carotenoids using HPLC-UV

Carotenoids were analysed as previously reported, with minor modifications (Cho et al., 2007). Briefly, 100 mg lyophilised sample was used for carotenoid analysis. Samples were incubated with methanol for 2 h at room temperature, followed by extraction with THF at least 4 times. The lipid-soluble phytonutrients were quantified using high-performance liquid chromatography (HPLC) with a C30 column (3 μm, 150 × 4.6 mm, YMC, Wilmington, NC). Carotenoids were monitored at 455 nm, with a Waters 2996 photo-diode array detector (Milford, MA).

HPLC mobile phases were methanol:methyl *tert*-butyl ether (MTBE):water (95:3:2 by vol with 1.5% ammonium acetate in water) for solvent **A** and methanol:MTBE:water (8:90:2 by vol with 1.0% ammonium acetate in water) for solvent **B**. The mobile phase gradient, at a flow rate of 0.4 ml min^{-1} (10 °C), was: (1) start at 100% solvent **A**; (2) a 21 min linear gradient to 45% solvent **A**; (3) 1 min hold at 45% solvent **A**; (4) an 11 min linear gradient to 5% solvent **A**; (5) a 4 min hold at 5% solvent **A** B; (6) a 2 min linear gradient back to 100% solvent **A**; (7) a 28 min hold at 100% solvent **A**. All-*trans*-β-carotene, 9-*cis*-β-carotene, α-carotene, β-cryptoxanthin, lutein, zeaxanthin, *cis* and *trans*-lycopene, and α- and γ-tocopherol were adequately separated and quantified using this method. Lipid-soluble phytonutrients were quantified by determining peak areas under the curve in the HPLC calibrated against known amounts of standards. Identification of each peak in unknowns was confirmed by the retention time and characteristic spectra of the standards. The inter-assay coefficient of variation (CV) was 4% ($n = 25$) and the intra-assay CV was 4% ($n = 9$).

2.4.2. Determination of ascorbic acid using a HPLC-ECD

Ascorbic acid was extracted as previously reported, with minor modifications (Behrens & Madere, 1987). The lyophilised sample (100 mg) was used for ascorbic acid analysis using an HPLC with Millennium 32 software (Waters Associates, Inc.) and BAS EC-5 ECD (Bioanalytical Systems Inc., West Lafayette, IN).

2.4.3. Determination of total phenols using a spectrophotometer

Phenolics were sequentially extracted twice with acidified methanol (methanol:glacial acetic acid:water, 50:3.7:46.3) from 100 mg lyophilised samples over a 16 h period. The combined extract was dried under nitrogen gas, and stored under nitrogen at −80 °C. Immediately before determination of total phenol content, the dry residue was reconstituted with water. The total phenol content was assayed in duplicate using Folin–Ciocalteu reagent, according to the method of Singleton, Orthofer, and Lamuela-Raventos (1999). Absorbance was determined at 725 nm using a Shimadzu UV1601 spectrophotometer (Kyoto, Japan). The total phenol content was calculated based on a standard curve established with authentic gallic acid standard. The detection limit for the assay was 16.1 μg ml^{-1}. The final results were expressed as mg gallic acid equivalents (GAE) g^{-1} dry weight. The inter-assay CV for the extraction and phenols determination from lyophilised samples was 3.3%.

2.4.4. Determination of selected phenolic acids and flavonoids using an HPLC-ECD

Dry acidified methanol extracts, obtained by the procedures described above for determination of total phenol content, were used for flavonoid and phenolic acid analysis. Prior to analysis by HPLC with electrochemical detection (ECD), aglycones in the dry extract were obtained after sugar cleavage by an acid-boiling treatment, as described by Zhang, Kou, Fugal, and McLaughlin (2004). Following solvent removal under nitrogen gas and reconstitution in HPLC mobile phase **A**, phenolic acids and flavonoids were analysed on a Zorbax SB-C18 column (4.6 × 150 mm, 3.5 μm) in a CoulArray 5600A (ESA Inc. Chelmsford, MA) as described previously (Chen, Milbury, Lapsley, & Blumberg, 2005). Limits of quantitation for phenolic acid and flavonoids determined by HPLC-ECD were 1 ng on column. The linearities of calibration curves of authentic standards with concentrations ranging from 0.01 to 2 ng/ml were at least $\geqslant 0.991$.

2.4.5. Determination of polyphenols in Angelica keiskei using LC-ESI-MS/MS

LC-MS and LC-MS/MS experiments were performed using a Thermo-Finnigan LCQ Advantage ion trap mass spectrometer (Thermoquest, Milan, Italy) equipped with an ESI ion source (capillary temperature 220 °C, spray voltage 4.5 kV, and capillary voltage 3.0 V for negative and 43.0 V for positive-ion mode) and

connected to a quaternary pump HPLC (Surveyor LC, Thermoquest). For LC-MS analyses, the MS operated in both negative and positive-ion mode, with a scan range from *m/z* 150 to 800 at a scan rate of 0.5 scans/s. LC-MS/MS experiments were performed in a data-dependent scan mode, enabling dynamic exclusion under the following conditions: repeat count, 2; repeat duration, 0.5 min; exclusion duration, 2 min. For LC-MS/MS analysis, positive and negative-ion modes were run separately. HPLC separation was done by reverse phase elution (Synergi Max-RP 80 Å column, 150 × 2 mm; Phenomenex, Torrance, CA) under the following conditions: linear gradient from 95% **A** (0.1% HCOOH) to 100% **B** (CH_3CN:HCOOH 99.9:0.1% v:v) in 45 min. UV detection was performed with a photo-diode array detector (DAD) (Thermoquest) using a 200–600 nm scan range and a rate of 0.5 scan/s.

2.4.6. Determination of antioxidant activity using ORAC and TAP assays and fluorescence microplate readers

2.4.6.1. Oxygen-radical absorbance capacity (ORAC) assay. The acidified methanol extracts used for total phenols analysis were also used for the ORAC assay. Dry residue produced from acidified methanol extraction was reconstituted with phosphate-buffered saline (PBS), and then the hydrophilic antioxidant activity of sample was immediately determined using the ORAC assay. The ORAC assay was conducted according to Ou, Hampsch-Woodill, and Prior (2001), and Cao, Alessio, and Cutler (1993). Briefly, the area under the curve of the oxidation of fluorescein ($\lambda_{excitation}$ = 485 nm, $\lambda_{emission}$ = 520 nm) by peroxyl radicals generated by 2,2′-azobis (2-amidinopropane) dihydrochloride was determined. The assay was carried out on a FLUOstar OPTIMA plate reader (BMG LABTECH, Offenburg, Germany). The results were expressed as μmol Trolox Equivalents (TE) g^{-1} dry weight. The inter-assay CV was 7.3%.

2.4.6.2. Total antioxidant performance (TAP) assay. The TAP assay was conducted as previously described (Li et al., in press). Briefly, two hundred milligrams of lyophilised sample were used to extract the water- and fat-soluble components. Samples were incubated with 10 ml acidified methanol (methanol:glacial acetic acid:water, 50:3.7:46.3) for 30 min in a shaking water bath. Following centrifugation, 10 ml supernatant was dried under nitrogen gas, dissolved in 2 ml PBS (50 mM, pH 7.4), and set aside. The remaining residue from the acidified methanol extraction was re-extracted 4-times with 10 ml THF each. Two millilitres of combined THF extract were dried under nitrogen gas, dissolved in 0.5 ml of a Tween-60/chloroform solution (3 mg/ml), dried under nitrogen gas, and resuspended in 1 ml PBS solution, which was set aside.

Antioxidant activity was measured fluorometrically, using lipophilic radical initiator MeO-AMVN and lipophilic probe BODIPY. For BODIPY incorporation, 25 μl BODIPY stock solution (2 mM) was diluted 100-fold with PBS. An aliquot of 100 μl BODIPY/PBS was added to 200 μl samples, vortexed for 20 s at the lowest speed and incubated for 10 min at 37 °C. Then 15 μl MeO-AMVN was added to the sample and diluted to a final volume of 1 mL with PBS, yielding BODIPY and MeO-AMVN at a final concentration of 2 μM and 2 mM, respectively. A 200 μl aliquot of the resulting reaction mixture was transferred to a 96-microwell plate, and oxidation kinetics monitored *via* the fluorescence of the oxidation products of BODIPY with excitation wavelength at 485 nm and emission wavelength at 535 nm (Aldini, Yeum, Russell, & Krinsky, 2001). The results are expressed as the percentage of inhibition of BODIPY oxidation with respect to that of a control sample.

The control sample was prepared as follows: soybean phosphatidylcholine was dissolved in chloroform (3 mg/ml) and evaporated slowly under a stream of nitrogen gas. A thin lipid film was formed on the vessel wall by rolling the vial. The vial was then maintained under a nitrogen gas stream for an additional 20 min to eliminate residual solvent. The lipid film was then rehydrated with PBS (40 mM, pH 7.4) to a concentration of 3 mg/ml phospholipid, sonicated in a water bath (4 cycles of 10 s), aliquoted to 500 μl each, and stored at −80 °C.

2.5. Statistical analyses

The data were expressed as mean ± standard deviation (SD). One-way ANOVA and the Turkey's Honest Significant Difference test were performed to identify differences amongst groups, using SAS (Version 9.1, SAS Institute Inc., Cary, NC). Statistical significance was set at $p < 0.05$.

3. Results and discussion

*3.1. Phytonutrient contents and total antioxidant activity of **Angelica keiskei***

Fig. 1A shows the mean carotenoid content in three samples of *Angelica keiskei*. The major carotenoids in *Angelica keiskei* in a dried powder form were lutein and all *trans*-β-carotene. *Angelica keiskei* **1** had the highest lutein and *trans*-β-carotene of 265 ± 11.5 and 130 ± 6.0 mg/kg dry weight, followed by *Angelica keiskei* **2** (252 ± 9.6, 117 ± 4.9 mg/kg dry weight) and *Angelica keiskei* **3** (205 ± 8.6, 103 ± 5.5 mg/kg dry weight). The carotenoid contents in *Angelica keiskei* were lower than in a previous report (Cho et al., 2007), probably due to the different part of *Angelica keiskei* used and a different drying process. The current study used leaves, stems and branches of *Angelica keiskei* and dried at 45–48 °C after freezing at −80 °C, whereas Cho et al. selected only leaves of *Angelica keiskei*, which underwent freeze drying.

As shown in Fig. 1B, *Angelica keiskei* **3** had 1.0 g/kg of ascorbic acid, twice that of the other two varieties. Quercetin and ferulic acid were the major compounds amongst 8 selected flavonoids and phenolic acids determined (Table 1). In *Angelica keiskei* **3**, grown in a greenhouse, total phenols reached 9.77 g/kg. Various factors, such as climate and sunlight exposure, probably all

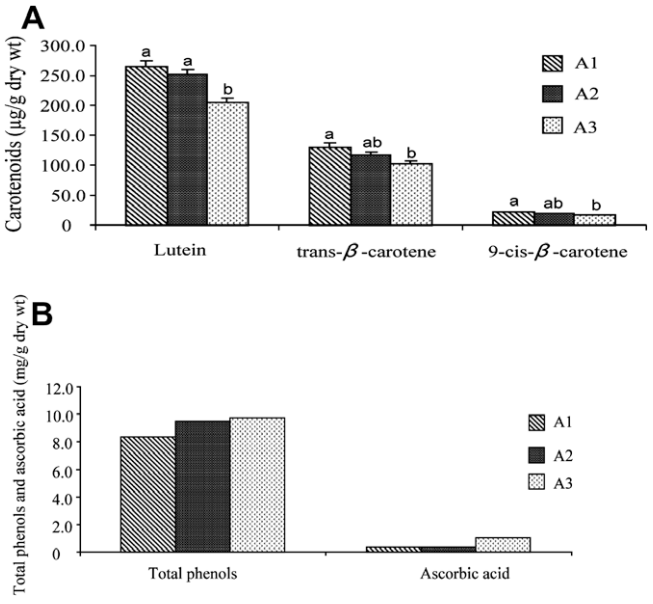

Fig. 1. Lipid-soluble (A) and water-soluble (B) phytonutrient concentrations in *Angelica keiskei* grown in three different conditions. A1: *Angelica keiskei* 1 greenhouse grown in central Korea, A2: *Angelica keiskei* 2 harvested from a farm in southern Korea, A3: *Angelica keiskei* 3 greenhouse grown in southern Korea. Samples were analysed in triplicate for carotenoids and duplicate for ascorbic acid and total phenols. The data are expressed as mean ± SD. Values with a different letter are significantly different ($p < 0.05$).

Table 1
Selected phenolic acids and flavonoids in *Angelica keiskei* grown in three different conditions (mg/kg dry weight).

	A1	A2	A3
Syringic acid	3.45	2.32	2.75
Epicatechin	2.33	3.90	9.82
p-Coumaric acid	7.59	10.2	19.3
Ferulic acid	10.4	19.3	23.8
Resveratrol	3.29	2.48	1.86
Quercetin	115	167	57.4
Kaempferol	4.14	8.99	1.31
Isorhamnetin	0.87	2.26	0.58

Whole *Angelica keiskei* dried powder samples were analysed in duplicate. A1: *Angelica keiskei* **1** greenhouse grown in central Korea; A2: *Angelica keiskei* **2** harvested from a farm in southern Korea; A3: *Angelica keiskei* **3** greenhouse grown in southern Korea.

contributed to the variation of phytonutrients in these three different samples of *Angelica keiskei*.

The antioxidant activities determined by ORAC and TAP are shown in Fig. 2. Both ORAC and TAP values tended to be highest in *Angelica keiskei* **3**, which has the highest total phenol content. In accordance with our previous report (Cho et al., 2007), total phenols and ORAC values were well correlated, since both assays used the same acidified methanol extract. *Angelica keiskei* **3** had the highest TAP value, which is related to the hydrophilic and lipophilic antioxidants and their interactions, suggesting a relatively minor contribution of carotenoids to the total antioxidant activity of *Angelica keiskei*, as well as a significant contribution of lipophilic phenolic components. It is also probable that other lipophilic phytonutrients as well as hydrophilic antioxidants contributed to the TAP of *Angelica keiskei*.

3.2. Identification and characterisation of polyphenols in Angelica keiskei

The UV profile of the 70% ethanol extract of *Angelica keiskei* indicated five major peaks and several minor peaks, eluting between 34 and 40 min. The peak eluting at 21.15 min showed a UV spectrum typical of caffeic acid derivatives (absorption band at 328 and 242 nm, with a diagnostic shoulder at 305 nm). The UV spectra of peaks eluting at 23.17 and 23.55 min, with λ_{max} at 340–355 nm (Band **I**) and 240–255 nm (Band **II**), shoulder at 300 nm and minimum at 280 nm, suggested flavone or flavonol glycosides. Peaks eluting at 43.38 and 43.97 min were characterised by λ_{max} values at 370 and 240 nm, indicating a chalcone moiety.

The full scan negative- and positive-ion mass spectra of the components of the *Angelica keiskei* extract allowed the definition of molecular masses. Each compound gave reasonably intense $[M-H]^-$ and $[M+H]^+$ ions, which provided some indication of their structure, further confirmed by MS/MS analyses carried out in data-dependent scan mode. The peak eluting at 21.15 min was characterised by a MW of 354 and fragmentation patterns in negative-ion mode with ions at *m/z* 179 [caffeic acid−H]$^-$ and *m/z* 191 [quinic acid−H]$^-$, which were confirmed in positive-ion mode with ions at *m/z* 193 [quinic acid+H]$^+$ and at *m/z* 163 [caffeic acid+H−H_2O]$^+$. The UV, MS and MS/MS data were consistent with the structure of chlorogenic acid (MW 354.3), as confirmed by an LC/MS match obtained with authentic standard. The peak eluting at 23.17 min was characterised by a MW of 594 and the MS/MS spectrum recorded in negative-ion mode showed a base peak at *m/z* 285 [M−H−Rha−Glu] and a less abundant ion (5%) at *m/z* 447 [M−H−Rha]. The fragment ions were also confirmed in positive-ion mode showing the base peak at *m/z* 449 [M+H−Rha]$^+$ and a minor peak at *m/z* 287 [M+H−Rha−Glu]$^+$. This peak was assigned the structure of luteolin diglycoside and the sequential loss of rhamnosyl and glucosyl residues indicated a diglycosyl moiety with a terminal desoxyhexosyl unit. In the peak at 23.55 min, two different compounds eluted, since the MS spectrum recorded in negative-ion mode showed two different $[M-H]^-$ ions at *m/z* 463 and *m/z* 447. The MS spectrum recorded in positive-ion mode confirmed the MW (ions at *m/z* 465 and *m/z* 449) and it was also characterised by two ions at *m/z* 287 and 303. MS/MS experiments

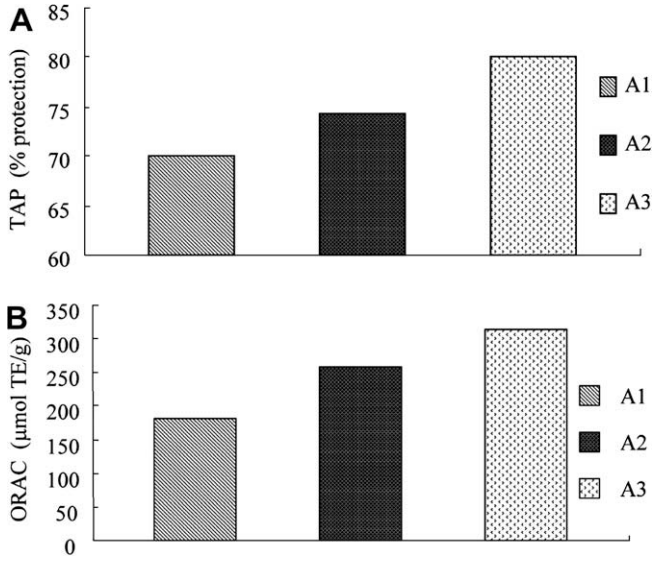

Fig. 2. Total antioxidant performance (A) and oxygen radical absorbance capacity (B) in *Angelica keiskei* grown in three different conditions. A1: *Angelica keiskei* 1 greenhouse grown in central Korea, A2: *Angelica keiskei* 2 harvested from a farm in southern Korea, A3: *Angelica keiskei* 3 greenhouse grown in southern Korea. Total antioxidant performance (TAP) is expressed as % protection with respect to control solution (phosphatidylcholine liposomes in PBS) and oxygen radical absorbance capacity is expressed as μmol tocopherol equivalent (TE)/g. Samples were analysed in triplicate for TAP and duplicate for ORAC. The data are expressed as mean ± SD.

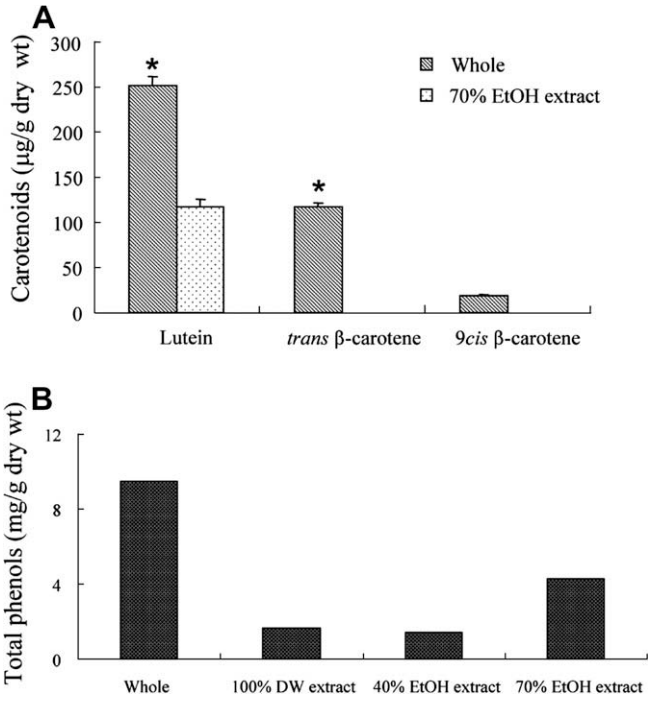

Fig. 3. Carotenoids (A) and total phenol (B) concentrations in whole powder and different extracts of *Angelica keiskei*. Samples were analysed in triplicate for carotenoids. The data are expressed as mean ± SD. Values with a marker are significantly different ($p < 0.05$). Total phenols are expressed as mg/g dry weight of duplicate analyses.

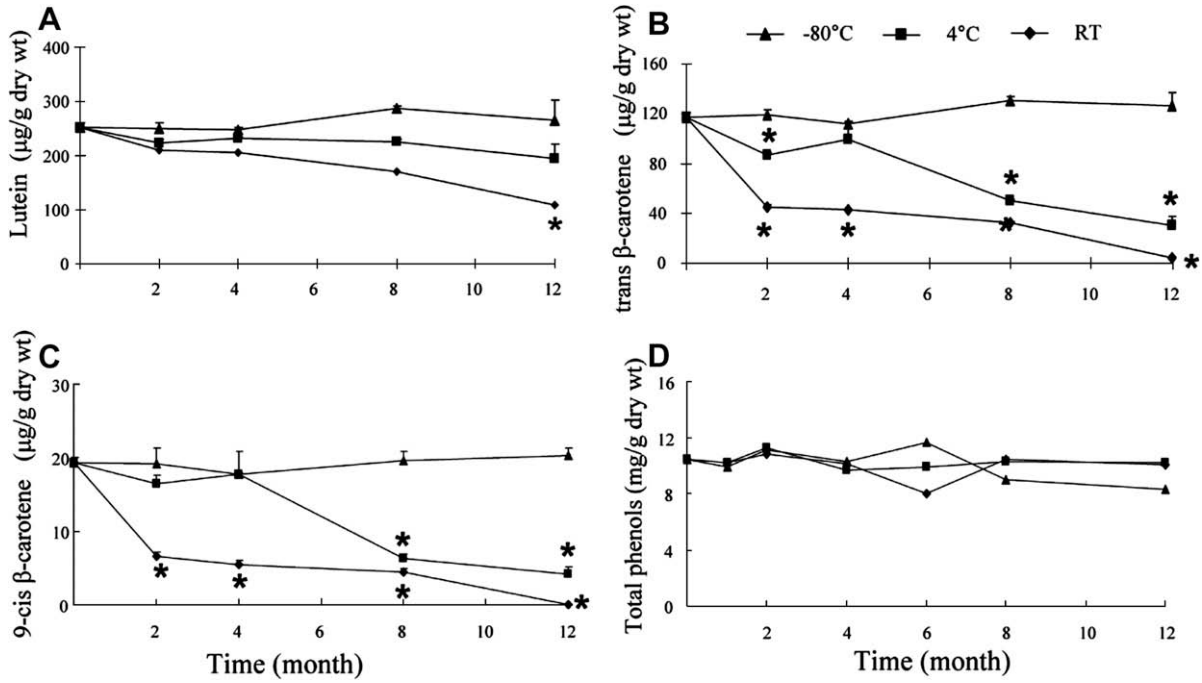

Fig. 4. Carotenoids and total phenols in *Angelica keiskei* after 12 months of storage at room temperature (22 °C), 4 °C and −80 °C. A: lutein B: all-*trans*-β-carotene C: 9-*cis*-β-carotene D: total phenols. Samples were analysed in triplicate. The data are expressed as mean ± SD. * ($p < 0.05$) Significantly different from the baseline. Total phenols are expressed as mg/g dry weight of duplicate analyses.

confirmed that both compounds were glycosides (Fig. 4; peaks c1, c2) and were assigned to luteolin *O*-glucoside (MW 448.38) and quercetin *O*-glucoside (MW 464.38). Luteolin *O*-glucoside can be tentatively identified as cynaroside (luteolin 7-glucoside), previously identified in the air-dried aerial parts of *Angelica keiskei* (Chen, 2004).

Peaks eluting at 43.38 and 43.97 min possessed molecular weights of 338 and 392 and based on the typical UV spectra of chalcones, they were assigned to the two main chalcones present in *Angelica keiskei*, 4-hydroxyderricin (MW 338.40) and xanthoangelol (MW 392.49), in accordance with a previous report (Ogawa, Nakashima, & Baba, 2003). It is interesting to note that these chalcones were reported to show beneficial effects on blood pressure, lipid metabolism (Ogawa, Okada, Kamisako, & Baba, 2007), as well as controlling blood glucose (Enoki et al., 2007) in rodent models.

3.3. Phytonutrients of Angelica keiskei in different extracts

Since carotenoids are lipophilic, it is not surprising that no carotenoids were detected in 100% water extract of *Angelica keiskei*, as shown in Fig. 3. Even 70% ethanol can only extract 46% of lutein in *Angelica keiskei*, indicating poor extraction efficiency of ethanol/water for carotenoids. Forty-five percent of total phenols in *Angelica keiskei* was extracted by 70% ethanol (4.28 mg/g), which was twice as high as for 40% ethanol and 100% water extracts.

The low yield of lutein and total phenols in *Angelica keiskei* by water/ethanol extraction may be due to the low extraction efficiency (Li, Jiang, & Chen 2002) and possible loss of phytonutrients during the extraction procedures, as reported by Howe and Tanumihardjo (2006).

3.4. Stability of phytonutrients in Angelica keiskei after 12 months of storage at room temperature, 4 °C and −80 °C

Angelica keiskei is a rich source of phytonutrients. However, the stability of phytonutrients and their antioxidant activity could be an important issue in plant foods. In the current study, Fig. 4 indicates no significant decrease in lutein content of *Angelica keiskei* stored at 4 °C and −80 °C up to 12 months. On the other hand, lutein degraded significantly after 12 months of storage at room temperature (Fig. 4A). All *trans*-β-carotene (Fig. 4B) and 9-*cis*-β-carotene (Fig. 4C) in *Angelica keiskei* stored at 4 °C decreased 74% and 63%, respectively, as compared to those of baseline. In addition, these carotenoids were almost completely degraded after 12 months of storage at room temperature. No significant degradation of phytonutrients was found in *Angelica keiskei* stored at −80 °C.

As shown in Fig. 4D, no significant decrease of total phenols was found in *Angelica keiskei* stored at room temperature, 4 °C and −80 °C for up to12 months. Our results are in accordance with Connor's study on fresh blueberry (Connor, Luby, Hancock, Berkheimer, & Hanson, 2002), showing no change in total phenols in blueberry stored at 5 °C for 5 weeks.

4. Conclusions

The current study clearly indicates that *Angelica keiskei* is a rich source of phytonutrients. In particular, LC-ESI-MS/MS analysis identified chalcones as abundant phytonutrients in *Angelica keiskei,* which are reported to possess antidiabetic and hypotriglyceridemic activities in animal models. Due to the poor extraction efficiency of phytonutrients in *Angelica keiskei* by water and ethanol, whole dry powder of *Angelica keiskei,* which can be kept stable for an extended period of time at low temperature, may be a good option for chronic intervention studies or for dietary supplements for humans.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2008.12.015.

References

Aaby, K., Wrolstad, R. E., Ekeberg, D., & Skrede, G. (2007). Polyphenol composition and antioxidant activity in strawberry purees; impact of achene level and storage. *Journal of Agriculture and Food Chemistry, 55*(13), 5156–5166.

Aldini, G., Yeum, K. J., Russell, R. M., & Krinsky, N. I. (2001). A method to measure the oxidizability of both the aqueous and lipid compartments of plasma. *Free Radical Biology and Medicine, 31*(9), 1043–1050.

Behrens, W. A., & Madere, R. (1987). A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic and dehydroascorbic acid in tissues, biological fluids, and foods. *Analytical Biochemistry, 165*(1), 102–107.

Cao, G., Alessio, H. M., & Cutler, R. G. (1993). Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine, 14*(3), 303–311.

Chen, C.-Y. (2004). Trace elements in Taiwanese health food, *Angelica keiskei*, and other products. *Food Chemistry, 84*, 545–549.

Chen, C. Y., Milbury, P. E., Lapsley, K., & Blumberg, J. B. (2005). Flavonoids from almond skins are bioavailable and act synergistically with vitamins C and E to enhance hamster and human LDL resistance to oxidation. *Journal of Nutrition, 135*(6), 1366–1373.

Cho, Y.-S., Yeum, K.-J., Chen, C.-Y., Beretta, G., Tang, G., Krinsky, N. I., et al. (2007). Phytonutrients affecting hydrophilic and lipophilic antioxidant activities in fruits, vegetable and legumes. *Journal of the Science of Food and Agriculture, 87*, 1096–1107.

Connor, A. M., Luby, J. J., Hancock, J. F., Berkheimer, S., & Hanson, E. J. (2002). Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage. *Journal of the Science of Food and Agriculture, 50*(4), 893–898.

Enoki, T., Ohnogi, H., Nagamine, K., Kudo, Y., Sugiyama, K., Tanabe, M., Kobayashi, E., et al. (2007). Antidiabetic activities of chalcones isolated from a Japanese Herb, *Angelica keiskei*. *Journal of the Science of Food and Agriculture, 55*(15), 6013–6017.

Fujita, T., Sakuma, S., Sumiya, T., Nishida, H., Fujimoto, Y., et al. (1992). The effects of xanthoangelol E on arachidonic acid metabolism in the gastric antral mucosa and platelet of the rabbit. *Research Communications in Chemical Pathology and Pharmacology, 77*(2), 227–240.

Howe, J. A., & Tanumihardjo, S. A. (2006). Evaluation of analytical methods for carotenoid extraction from biofortified maize (*Zea mays* sp.). *Journal of Agriculture and Food Chemistry, 54*(21), 7992–7997.

Li, L., Chen, C. -Y., Chun, H. -K., Cho, S. -M., Park, K. -M., Lee-Kim, Y. C., et al., (in press). A fluorometric assay to determine antioxidant activity of both hydrophilic and lipophilic components in plant foods. *Journal of Nutritional Biochemistry*, doi:10.1016/j.jnutbio.2008.02.006.

Li, H. B., Jiang, Y., & Chen, F. (2002). Isolation and purification of lutein from the microalga *Chlorella vulgaris* by extraction after saponification. *Journal of Agriculture and Food Chemistry, 50*(5), 1070–1072.

Matsuura, M., Kimura, Y., Nakata, K., Baba, K., & Okuda, H. (2001). Artery relaxation by chalcones isolated from the roots of *Angelica keiskei*. *Planta Medica, 67*(3), 230–235.

Murakami, S., Kijima, H., Isobe, Y., Muramatsu, M., Aihara, H., Otomo, S., et al. (1990). Inhibition of gastric H+, K(+)-ATPase by chalcone derivatives, xanthoangelol and 4-hydroxyderricin, from *Angelica keiskei* Koidzumi. *Journal of Pharmacy and Pharmacology, 42*(10), 723–726.

Ogawa, H., Nakashima, S., & Baba, K. (2003). Effects of dietary *Angelica keiskei* on lipid metabolism in stroke-prone spontaneously hypertensive rats. *Clinical and Experimental Pharmacology and Physiology, 30*(4), 284–288.

Ogawa, H., Okada, Y., Kamisako, T., & Baba, K. (2007). Beneficial effect of xanthoangelol, a chalcone compound from *Angelica keiskei*, on lipid metabolism in stroke-prone spontaneously hypertensive rats. *Clinical and Experimental Pharmacology and Physiology, 34*(3), 238–243.

Okuyama, T., Takata, M., Takayasu, J., Hasegawa, T., Tokuda, H., Nishino, A., et al. (1991). Anti-tumor-promotion by principles obtained from *Angelica keiskei*. *Planta Medica, 57*(3), 242–246.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*(10), 4619–4626.

Rangkadilok, N., Worasuttayangkurn, L., Bennett, R. N., & Satayavivad, J. (2005). Identification and quantification of polyphenolic compounds in Longan (Euphoria longana Lam.) fruit. *Journal of Agricultural and Food Chemistry, 53*, 1387–1392.

Shimizu, E., Hayashi, A., Takahashi, R., Aoyagi, Y., Murakami, T., & Kimoto, K. (1999). Effects of angiotensin I-converting enzyme inhibitor from Ashitaba (*Angelica keiskei*) on blood pressure of spontaneously hypertensive rats. *Journal of Nutritional Science and Vitaminology (Tokyo), 45*(3), 375–383.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.

Zafrilla, P., Ferreres, F., & Tomas-Barberan, F. A. (2001). Effect of processing and storage on the antioxidant ellagic acid derivatives and flavonoids of red raspberry (*Rubus idaeus*) jams. *Journal of Agricultural and Food Chemistry, 49*(8), 3651–3655.

Zhang, Z., Kou, X., Fugal, K., & McLaughlin, J. (2004). Comparison of HPLC methods for determination of anthocyanins and anthocyanidins in bilberry extracts. *Journal of Agricultural and Food Chemistry, 52*(4), 688–691.