Summary
The seeds of nine China-grown radish cultivars were analyzed for their phytochemical composition, antioxidant properties and ACE-inhibitory activity. Radish seeds contained 36.87-43.06% (w/w) oils, whereas 64.55-69.26% of the fatty acids were monounsaturated and 20.33-25.11% were polyunsaturated. The levels of δ-tocopherol (552.24-670.31 μg/g seed oils) and lutein (4.82-8.95 μg/g seed oils) differed in cultivars. The nine cultivars varied in total phenolics, flavonoids, and free phenolic acids, but not in proanthocyanidins. Seed extracts of Hybrid #63, Tou Xin Hong, and Hybrid #72 showed stronger DPPH radical scavenging capacity, ORAC, and FRAP than others (p<0.05). The image showed a strong effect of antioxidant activity, which was positively correlated with vanillic acid contents (r = 0.890, p = 0.001). It provides evidence on developing value-added utilization of radish seeds or seed fractions such as oil and flour as nutraceuticals or functional food ingredients.

Keywords: Radish seeds; Phenolics; Flavonoids; Antioxidants; ACE-inhibitory activity

Introduction
Radish (Raphanus sativus L.) is a root vegetable crop that is native to Europe and Eastern Asia (Muminovic et al., 2005). The consumption of Radish has increased during the last decade due in part to recognition of their nutritional values and antioxidant properties (Lugasi et al., 1998; Vitória et al., 2001). It reported that radish consumption could reduce the risk of lung and colorectal cancers (Martinez-Villaluenga et al., 2010), and minimize genotoxicity and cytotoxicity (Hassan et al., 2011).

The radish seeds have gained considerable attention of countries such as Brazil, Turkey and Poland, due to their high levels of unsaturated fatty acids (Uluta and Özdemir, 2012; Avila and Sodré, 2012; Kaymak, 2015) and other antioxidant compounds, such as phenolic acids and flavonoids (Pajić et al., 2014). So, the extracts from the radish seeds or seed flour could be potent functional food ingredients. A recent study reported that total seed oil (43%, w/w) of Turkey-grown radish consists of 62% monounsaturated fatty acids (MUFA's) and 17% polyunsaturated fatty acids (PUFA's) and radish seed oil contains significantly high levels of tocopherols (Uluta and Özdemir, 2012). Compared to selected seeds including mung bean, broccoli, and sunflower seeds, radish seeds in Poland contain higher levels of total phenolics and flavonoids (Pajić et al., 2014). Additionally, radish seeds contain functional proteins with in vitro antifungal activity (Terras et al., 1992).

About 17.5 million people die each year from cardiovascular diseases (CVDs), an estimated 31% of all deaths worldwide (http://www.who.int/cardiovascular_diseases/en). Hypertension and chronic inflammation, both of which can be caused by oxidation in vivo, are regarded as risk factors of CVD. Many studies showed that the secondary metabolites from plants are the potential compounds for preventing CVD, possible attributable to their antioxidant and angiotensin-converting enzyme (ACE)-inhibitory activity (Vasanthi et al., 2012; Balasuriya and Rupasinghe, 2011). Lopizzo et al. (2007) isolated six flavonoids with ACE-inhibitory activity from Allianthus excelsa (Roxb), of which kaempferol-3-O-β-galactopyranoside possesses the highest activity with an IC50 value of 260 μmol/L. Oeda et al. (2010) isolated and identified two ACE inhibiting anthocyanins, delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside, from the aqueous extract of Hibiscus sabdariffa with IC50 of 84.5 and 68.4 g/mL, respectively. Most of the researches have been targeted at bioactive compounds from natural resources. It is almost universally accepted that the phytochemicals are the new alternatives for replacing the drugs to avoid the adverse side effects initiated by long use of the ACE inhibitors, such as captopril and Lisinopril.

There are several radish cultivars grown in different regions of China. White round, Korean white, Japan white, and White pink are the radish cultivars with high consumption, and Tou Xin Hong, Xin Lin Mei, and Yanzhi #2 are the radish cultivars whose roots are rich in pigments we have investigated (Jing et al., 2012), and Hybrid #63 and Hybrid #72 are new hybrid radish cultivars which have been created by Prof. Pan. However, few studies have evaluated their phytochemical compositions and antioxidant activities in seeds. Therefore, the objective of this study was to investigate the levels of fatty acids, tocopherols, carotenoids, total phenolics, flavonoids, and proanthocyanidins in the seeds of these nine radish cultivars and to evaluate the antioxidant capacity and ACE-inhibitory properties of these radish seeds.

Materials and methods
Radish seeds and chemicals
The nine radish cultivar were cultivated with conventional plantation measure, and their seeds were harvested by Zhenjiang Institute of Agricultural Sciences in Hilly Area of Jiangsuo Province and donated to this experiment. Standards of Supelco 37 Component FAME Mix, α, β, γ, δ-tocopherols, β-carotene, lutein, cryptoxanthin, and zeaxanthin, 2,4,6-tripyridyl-S-triazine(TPTZ), 6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocalteu reagent, 2,2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diannmonium salt (ABTS), and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), Angiotensin-Converting Enzyme (ACE, EC 3.4.15.1) from rabbit lung, Hippuryl-His-Leu (HHL), and sinapic, vanillic, syringic, and ferulic

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Phytochemical composition, antioxidant capacity and ACE-inhibitory activity of China-grown radish seeds
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acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid, (±)-catechin hydrate, cyanidin chloride were purchased from Aladdin (Shanghai, China). All other chemicals and solvents were of the highest commercial grade and used without further purification. All other chemicals were purchased from Sinopharm Chemical Reagent (Shanghai, China).

**Extraction of radish seeds**

The oils and antioxidant extracts were prepared according to the previous description by Jing et al. (2012). Radish seeds were ground into powder with a standard household coffee grinder until they could pass through a 40-mesh screen. Five grams of radish seed powder were extracted in 80 mL hexane for 6 h in a Soxhlet device. The hexane in the oil was evaporated using a rotary evaporator (Rotavapor RE-52, Yarong Inc., Shanghai, China) at reduced pressure. The oils were collected and stored at -20°C until analysis. Each sample was analyzed in three technical repeats. The radish seed flour (RSF) after oil extraction was then dried overnight under hood and weighed, where the weight loss was equal to the mass of extracted oils.

One gram of the flour was shaking in water bath with 10 mL of 50% acetone at room temperature for 2 h. Then, the extracts were centrifuged at 3000 g for 8 min. The prepared extracts were kept in the dark at 4°C for further analysis of antioxidants, such as phenolic acid, flavonoids, and proanthocyanidins. Each sample was analyzed in three technical repeats.

**Analysis of FA composition by gas chromatography (GC)**

Fatty acid methyl esters (FAME) were prepared by the KOH–methanol method (Jing et al., 2012) prior to analysis of GC. Briefly, dissolving 20 mg oil in 2 mL iso-octane followed by another addition of 0.2 mL of 1 mol/L KOH–methanol. After a reaction at room temperature for 5 min, 2 mL of iso-octane and approximately 3 mL water were successively added. Then, removing the supernatant and washing the residues with 3 mL water twice. The upper iso-octane layer was collected and subjected to fatty acid analysis using gas chromatography (Shimadzu GC-2010), which was equipped with an Omegawax column (30 m × 0.25 mm with a 0.25 μm film thickness) from Supelco (Bellefonte, PA, USA) and a flame ionization detector (FID). The carrier gas was Helium, and the flow rate was set as 1.0 mL/min. Oven temperature-rising program was set as follows: initially 50°C for 2 min, increasing to 220°C at 30°C/min and holding at 220°C for 20 min, followed by heating from 220 to 260°C at 10°C/min and holding at 260°C for 10 min. Each sample was analyzed in three technical repeats. The various fatty acids were identified according to the standards mixture (Supelco 37 Component FAME Mix, Supelco, PA, USA). The concentration of each fatty acid was expressed as a percentage of total area of all fatty acid peaks.

**Determination of tocopherol contents of oil from seed by Ultra Performance Liquid Chromatography (UPLC)**

The separation samples were prepared by dissolving about 200 mg oil in 10 mL methanol/tetrahydrofuran (THF) (1:1, v/v/v) prior to analysis of tocopherol contents in seed oil. The samples were separated on a Waters ACQUITY UPLC-C18 column (100 mm × 2.1 mm, 1.7 μm particle size) using an ACQUITY Ultra Performance Liquid Chromatography (UPLC) system with a TUV detector (Waters, Milford, USA) according to a previous method (Jing et al., 2012). Elution was performed at a flow rate of 0.3 mL/min with a binary gradient (a mobile phase of water was used as solvent A, and acetonitrile was used as solvent B) of solvent B in A going from 80% to 25% over 10 min, from 95% to 100% for next 5 min. Tocopherols were monitored at 294 nm and identified according to the UPLC retention time with those of tocopherol standards. The standard curves were constructed for quantification. Each sample was analyzed in three technical repeats.

**Analysis of Carotenoid compositions by Ultra Performance Liquid Chromatography (UPLC)**

The separation samples prepared was also loaded on C-30 YMC carotenoid column (YMC, Wilmington, NC; 150 × 4.6 mm, 5 μm particle size) using the ACQUITY Ultra Performance Liquid Chromatography (UPLC) system equipped with a TUV detector according to a previous method (Jing et al., 2012). The mobile phase was methanol/methyl tertiary butyl ether (MTBE)/H2O (81:15:4, v/v/v) as solvent A and MTBE/methanol (91:9, v/v) as solvent B. Elution was performed at a flow rate of 1 mL/min with a binary gradient of solvent B in A going from 0% to 50% over 30 min, 100% for next 10 min, and 0% for next 5 min. Carotenoids were monitored at 450 nm and identified by comparing the UPLC retention time with standard (compounds containing β-carotene, lutein, cryptoxanthin, and zeaxanthin), and then quantified based on standard curves. Each sample was analyzed with three technical repeats.

**Determination of total phenolic contents (TPC) of radish seed flour extract**

Total phenolics of radish seed flour extract were measured using a modified Folin-Ciocalteau method (Waterhouse, 2001). Briefly, 50 μL of radish seed flour extract samples, gallic acid dilutions (standards), or water blank was added into each tube, respectively, which was filled with 3 mL of water and 250 μL of Folin-Ciocalteau reagent in advance. The sample were mixed well and placed at ambient temperature for 10 min. Then, 750 μL of 20% (w/v) Na2CO3 solution was added into each test tube and mixed well prior to reaction at ambient temperature for 2 h. Absorbance was read at 765 nm using a LSS UV/Vis spectrophotometer (Shanghai Analytical Instrument, China). Each test was performed in triplicate. Total phenolics were calculated as gallic acid equivalents (GAE) per gram of radish seeds based on a gallic acid standard curve.

**Analysis of Phenolic acid composition by High Performance Liquid Chromatography (HPLC)**

Soluble free phenolic acid compositions in each radish seed were analyzed with a previously reported procedure (Jing et al., 2012). For quantitative analysis of phenolic acid composition in antioxidant extracts of radish seeds, the extracts were loaded on a Zorbax Eclipse XDB-C18 column (250 mm × 4.6 mm, 5 μm, Agilent Technologies, Palo Alto, CA, USA) using Agilent 1260 infinity HPLC system. Phenolic acids were separated at a flow rate of 1 mL/min with a binary gradient (mobile phase of formic acid/H2O (0.1:99.9, v/v) was used as solvent A and mobile phase of formic acid/acetonitrile (0.1:99.9, v/v) was used as solvent B) with going from 0% to 7% over 5 min; from 7% to 25% B over 40 min; from 25% to 45% over 10 min. Phenolic acids were identified by comparing the retention time and spectrum of peaks in the samples to that of the standards under the same HPLC conditions. Each sample was analyzed in three technical repeats. Quantification of each phenolic acid was determined using external standards and total area under each peak.
to stand for 6 min. Subsequently, 20 μL of a 10% AlCl₃ solution was added and allowed to stand for 6 min. Finally, 60 μL of 4% (w/v) NaOH solution were added into the mixture to stop the reaction for 15 min. Absorbance was measured at 510 nm using a microplate reader (Infinite F200 PRO; Tecan, Switzerland). Each test was performed in triplicate, and total flavonoids were calculated as catechin equivalents (CAE) of radish seed based on a catechin standard curve.

**Determination of total proanthocyanidin content (TPCC) of radish seed flour extracts**

The TPCC of radish seed flour extracts were determined using the HCl/n-butanol assay (PORTER et al., 1985). Briefly, 0.5 mL of seed flour extract was added into test tubes containing 6 mL of a 95% solution of n-butanol/HCl (95:5, v/v) and mixed well. Subsequently, 0.2 mL of a solution of 2% (w/v) NH₄Fe(SO₄)₂ in 2 mol/L HCl was added into the mixture and incubated for 40 min at 90 °C. The absorbance was measured at 550 nm in LSS UV/Vis spectrophotometer. TPCC was expressed as mg of cyanidin equivalents (CyE) per gram of radish seeds.

**Evaluation of antioxidant capacity by chemical assays**

**DPPH radical scavenging capacity**

Briefly, 100 μL of 0.2 mmol/L DPPH solution was mixed with 100 μL of radish seed flour 50% acetone extracts at different concentrations to initiate the reactions in each well of a 96-well plate. Absorbance at 515 nm was determined after 30 min of reaction in a microplate reader (Infinite F200 PRO; Tecan, Switzerland). The control consisted of 100 μL of solvent and the control consisted of 100 μL of solvent and 100 μL of 0.2 mmol/L DPPH. The DPPH radical-scavenging activity in the extracts was expressed as micromoles of Trolox equivalents per gram of seed.

**Determination of Oxygen Radical Absorbance Capacity (ORAC) assay**

Determination of ORAC of the studied compounds was performed according to the previous description (JING et al., 2014). Same as samples tested, Trolox standards were dissolved in 50% acetone. Other reagents were prepared in 75 mmol/L phosphate buffer (pH 7.4). Briefly, 30 μL of 20 μmol/L extracts (or 50% acetone for blank control) were mixed 225 μL fluorescein (81.63 nmol/L) in each well of a 96-well plate. Subsequently, the plate was covered and incubated at 37 °C for 20 min. To start reaction, 25 μL of 0.36 M 2,2’-azobis(2-amidinopropane) hydrochloride (AAPH) were added and mixed well with above mixture in each well. The fluorescence was recorded with an excitation wavelength of 355 nm and an emission wavelength of 460 nm. The fluorescence was recorded at excitation and emission wavelengths of 355 and 460 nm, respectively, in a microplate reader (Infinite F200 PRO; Tecan, Switzerland). The control and blank, water was added instead of samples and ACE, respectively. The inhibition rate was calculated as the following:

\[
\text{ACE inhibitory activity} \% = \left(1 - \frac{f_b - f_c}{f_b} \right) \times 100
\]

Where \(f_c\) is the fluorescence intensity of the test sample in the presence of the reaction mixture, \(f_b\) is the fluorescence intensity of blank sample in the absence of ACE, and \(f_c\) is the fluorescence intensity of buffer in the absence of the test sample. Each sample was analyzed in three technical repeats.

**Statistical analysis**

All results are expressed as the mean±SD. Univariate ANOVA among means of chemical contents, antioxidant activities, or ACE-inhibitory activities in nine radish seeds were performed by least significant difference (LSD) test in General Linear Model at the level of 0.05. Correlation among means was determined using a two-tailed Pearson correlation test. Statistics was analyzed using SPSS (version 14.0, SPSS Inc., Chicago, IL, USA).

**Results and discussion**

Total phenolics, flavonoids, and proanthocyanidins in radish seeds

The total phenolics, flavonoids, and proanthocyanidins levels in radish cultivars are shown in Tab. 1. Total phenolic levels varied from 9.15 mg (in Hybrid #63) to 14.54 mg (in White pink) gallic acid equivalents (GAE)/g dry mass (DM). Among the cultivars, White pink seeds had the highest total phenolic levels (14.54 mg GAE/g DM; p<0.05). In this study, the total phenolic levels obtained were higher than those previously reported in radish seeds of 6.7 or 6.1 mg GAE/g DM (PAJAK et al., 2014; AGUILERA et al., 2015) and in pomegranate seeds of 1.29-2.17 mg GAE/g DM (JENG et al., 2012). The differences in results could be attributed to the species, cultivars, growing conditions, genotypes, and extraction methods. The results revealed that the tested radish seeds had high levels of total phenolic compounds. The total flavonoids in radish seeds varied from 0.51-1.36 mg catechin equivalents (CAE)/g DM (Tab. 1). Tou Xin Hong seeds contained the highest level of total flavonoids (1.36 mg CAE/g DM) followed by White pink and Yanzhi #2 (~1.28 mg CAE/g DM; p>0.05). It has been reported that radish leaves contain >200 mg CAE/g DM flavonoids (Kim et al., 2014), which is considerably higher than the level present in radish seeds. Total proanthocyanidin levels...
The predominant MUFAs were erucic (C22:1, 34.53-40.32%) and oleic (C18:1, 16.20-21.30%) acids that were slightly higher than or comparable to the previous reports by ÜLUATA and ÖZDEMİR (2012) or KAYMAK (2015), who found 22.1-36.4%, or 40.83% erucic and 12.6-23.9%, or 19.08% oleic acids in radish seed oils, respectively. The levels of linoleic (C18:2, 10.14-14.28%) and linolenic (C18:3, 8.40-11.17%) acids as major PUFA were comparable to those as 10.09% and 7.02% for each in previous literatures (ÜLUATA and ÖZDEMİR, 2012; KAYMAK, 2015).

**Tocopherols and carotenoids**

Tocopherols and carotenoids were detected in seeds oils of tested radish cultivars in Tab. 3. The δ-tocopherol varied from 552.24-670.31 μg/g seed oils, whereas α-, γ-, and β-tocopherols were not detected. The level of δ-tocopherol in China-grown radish seeds was greater than those in Turkey-grown radish seeds reported as 545.67 μg/g seed oils by ÜLUATA and ÖZDEMİR (2012), who also found α-, γ-, and β-tocopherols as 12.41-28.66 μg/g seed oils in Turkey radish seeds. Tou Xin Hong (670.31 μg/g seed oils) contained greater amount of δ-tocopherol than other seven cultivars in this study (p<0.05).

Among the carotenoids, only lutein was detected with levels ranging from 4.82-8.95 μg/g seed oils. Hybrid #63 seeds among all cultivars found the greatest amount of lutein (p<0.05), comparable to one in soybeans of 1.6-14.8 μg/g seed oils (KANAMARU et al., 2006).

**Free phenolic acid composition**

The sinapic, vanillic, syringic, and ferulic acid were found in the seed oils of radish seed oils. Among the carotenoids, only lutein was detected with levels ranging from 4.82-8.95 μg/g seed oils. Hybrid #63 seeds contained the greatest amount of lutein (p<0.05), comparable to one in soybeans of 1.6-14.8 μg/g seed oils (KANAMARU et al., 2006).

**Values expressed as mean ± SD (n=3). Different letters within a column represent significant differences (p<0.05). GAE: gallic acid equivalents; CE: catechin equivalents; CyE: cyanidin equivalents**

**Oils were extracted for 6 h from 40-mesh ground seeds by Soxhlet extraction. Data expressed as mean and standard deviation (n=3). SFAs: saturated fatty acids. MUFAs and PUFAs represent monounsaturated fatty acids and polyunsaturated fatty acids, respectively. Different letters within a column represent significant differences (p<0.05).**

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**Tab. 1:** Total phenolics, flavonoids, and proanthocyanidins in radish seeds

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Phenolics (mg GAE/g)</th>
<th>Flavonoids (mg CE/g)</th>
<th>Proanthocyanidins (mg CyE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White round</td>
<td>11.60 ± 0.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.77 ± 0.10&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.15 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Korean white</td>
<td>9.34 ± 0.59&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.68 ± 0.06&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.12 ± 0.35&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Japanese white</td>
<td>9.84 ± 0.53&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.86 ± 0.07&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.82 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hybrid #63</td>
<td>9.15 ± 0.94&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.51 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.90 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hybrid #72</td>
<td>9.93 ± 0.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.90 ± 0.17&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.79 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>White pink</td>
<td>14.54 ± 2.76&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.28 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tou Xin Hong</td>
<td>10.79 ± 0.61&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>1.36 ± 0.10&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.10 ± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Xin Lin Mei</td>
<td>10.51 ± 0.23&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.61 ± 0.06&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.74 ± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yanzhi #2</td>
<td>12.21 ± 0.81&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.28 ± 0.10&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.11 ± 0.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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**Tab. 2:** Fatty acid composition and total oil content of radish seeds

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Percentage of fatty acids (%)</th>
<th>Total oil (g/100g seeds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16:0</td>
<td>16:1</td>
</tr>
<tr>
<td>White round</td>
<td>4.82</td>
<td>0.18</td>
</tr>
<tr>
<td>Korean white</td>
<td>4.67</td>
<td>0.17</td>
</tr>
<tr>
<td>Japanese white</td>
<td>4.50</td>
<td>0.15</td>
</tr>
<tr>
<td>Hybrid #63</td>
<td>4.62</td>
<td>0.15</td>
</tr>
<tr>
<td>Hybrid #72</td>
<td>4.70</td>
<td>0.17</td>
</tr>
<tr>
<td>White pink</td>
<td>5.11</td>
<td>0.27</td>
</tr>
<tr>
<td>Tou Xin Hong</td>
<td>4.88</td>
<td>0.18</td>
</tr>
<tr>
<td>Xin Lin Mei</td>
<td>4.70</td>
<td>0.19</td>
</tr>
<tr>
<td>Yanzhi #2</td>
<td>4.72</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Antioxidant capacity

More than one radical system was applied to investigate the radical scavenging capacities of radish seed extracts and results are shown in Fig. 1. The 50% acetone extracts of radish seed flour (RSF) were used to quench DPPH radical in the testing system. The DPPH radical scavenging capacity of RSF from 14.84-26.35 μmol TE/g seeds in Fig. 1A, was much greater than those in radish seeds var. Flamboyant 2 (ferulic acid, 21 μg/g seeds) (PAJAK et al., 2014). Yanzhi #2 contained the highest levels of vanillic acid (18.77 μg/g seeds, p<0.05) among all tested seeds.

Tab. 3: Tocopherol and lutein contents of radish seed oils1

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>δ-Tocopherol (μg/g oils)</th>
<th>Lutein (μg/g oils)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White round</td>
<td>562.24 ± 61.27a</td>
<td>7.90 ± 0.34c</td>
</tr>
<tr>
<td>Korean white</td>
<td>579.92 ± 15.90a</td>
<td>6.20 ± 0.08b</td>
</tr>
<tr>
<td>Japan white</td>
<td>585.15 ± 47.90a</td>
<td>8.55 ± 0.35d</td>
</tr>
<tr>
<td>Hybrid #63</td>
<td>591.55 ± 46.92a</td>
<td>8.95 ± 0.31e</td>
</tr>
<tr>
<td>Hybrid #72</td>
<td>610.16 ± 32.94a</td>
<td>8.29 ± 0.10cd</td>
</tr>
<tr>
<td>White pink</td>
<td>656.60 ± 35.20b</td>
<td>5.98 ± 0.08b</td>
</tr>
<tr>
<td>Tou Xin Hong</td>
<td>670.31 ± 25.78b</td>
<td>8.31 ± 0.13d</td>
</tr>
<tr>
<td>Xin Lin Mei</td>
<td>578.25 ± 14.88a</td>
<td>7.55 ± 0.12c</td>
</tr>
<tr>
<td>Yanzhi #2</td>
<td>552.24 ± 25.97a</td>
<td>4.82 ± 0.06a</td>
</tr>
</tbody>
</table>

1Oils were extracted for 6 h from powder of radish seeds by the Soxhlet method. δ-tocopherol was quantified by HPLC. Results are expressed as μg/g oils. Values are expressed as mean±standard deviation (n = 3). Different letters within a column represent significant differences (p<0.05).

ACE-inhibitory activity

Angiotensin I converting enzyme (ACE) catalyzes the conversion of angiotensin I into angiotensin II, a strong vasoconstrictor that increases blood pressure (SKEEGS et al., 1956). Many compounds were reported to exert both of antioxidant activity and ACE-inhibitory activity (CHOPRA et al., 1992; PHILANTO et al., 2008). Therefore, the nine China-grown radish seeds were evaluated for their inhibitory effect on ACE, based on 1-mL extracts obtained from 2 mg of seed flour. Yanzhi #2 inhibited 100% of ACE activity, followed by Hybrid #63 (40.16%; Fig. 2). Additionally, plant flavonoids could promote RNA expression of endothelial nitric oxide synthase to increase the level of NO attributing to decreasing blood pressure (VASANTHI et al., 2012). So, it suggested that the extracts of RSF exhibited a potential role in promoting cardiovascular health.

Tab. 4: Free phenolic acids in radish seeds1

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Sinapic</th>
<th>Vanillic</th>
<th>Syringic</th>
<th>Ferulic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>White round</td>
<td>3.78±0.14a</td>
<td>ND</td>
<td>4.87±0.45ab</td>
<td>47.59±1.01c</td>
<td>56.24±1.57e</td>
</tr>
<tr>
<td>Korean white</td>
<td>3.62±0.45a</td>
<td>1.99±0.28b</td>
<td>6.94±0.70a</td>
<td>47.75±1.866</td>
<td>61.35±2.98e</td>
</tr>
<tr>
<td>Japan white</td>
<td>3.33±0.13a</td>
<td>1.32±0.08b</td>
<td>1.90±0.26ab</td>
<td>34.47±1.02c</td>
<td>40.92±1.49f</td>
</tr>
<tr>
<td>Hybrid #63</td>
<td>3.00±1.11a</td>
<td>ND</td>
<td>20.18±1.73c</td>
<td>23.18±1.84a</td>
<td></td>
</tr>
<tr>
<td>Hybrid #72</td>
<td>3.22±0.15a</td>
<td>0.46±0.01b</td>
<td>3.03±0.25ab</td>
<td>27.91±1.65b</td>
<td>34.62±2.06b</td>
</tr>
<tr>
<td>White pink</td>
<td>2.82±0.30a</td>
<td>1.29±0.05b</td>
<td>1.54±0.13b</td>
<td>33.40±1.42c</td>
<td>39.05±1.90b</td>
</tr>
<tr>
<td>Tou Xin Hong</td>
<td>3.65±0.12a</td>
<td>2.02±1.43b</td>
<td>3.73±0.12b</td>
<td>40.43±2.43d</td>
<td>49.83±2.81d</td>
</tr>
<tr>
<td>Xin Lin Mei</td>
<td>3.52±0.22a</td>
<td>1.25±1.25b</td>
<td>4.29±0.56b</td>
<td>35.70±2.31cd</td>
<td>41.24±3.21c</td>
</tr>
<tr>
<td>Yanzhi #2</td>
<td>ND</td>
<td>18.77±1.65a</td>
<td>4.55±0.43b</td>
<td>46.77±3.20e</td>
<td>70.09±5.28f</td>
</tr>
</tbody>
</table>

1Sinapic, vanillic, syringic, and ferulic stand for sinapic, vanillic, syringic, and ferulic acids. Results expressed as micrograms of individual standard per gram of radish seeds. Data expressed as mean±standard deviation (n = 3). Different letters within a column represent significant differences (p<0.05); ND, not detected.
There were no correlations between antioxidant activity and ACE-inhibitory activity, or with vanillic acid. Another report also described the similar results, in which intake of flavonoid-rich apple peel extract did not cause significant differences in serum and lung ACE activity at week eight but reduced blood pressure after 5 weeks of treatment in spontaneously hypertensive rats (SHR) possibly through endogenous antioxidant pathways (Balasuriya et al., 2015). The IC_{50} of vanillic acid for ACE-inhibitory activity was about 8 mmol/L, which linear correlated to the molecular docking score between phenolic compounds and testicular ACE (Al Shukor et al., 2013). The ACE-inhibitory activity of vanillic acid was proposed to be charge-charge interactions with the zinc ion in the active site of ACE (Al Shukor et al., 2013). Therefore, vanillic acid might be responsible for the ACE-inhibitory activity of radish seeds but not for their antioxidant activities like phenolics and flavonoids (Schewe et al., 2008; Vasanthi et al., 2012). Certainly, as mentioned in the introduction, some flavonoids and anthocyanins also had ACE inhibitoring activity (Loizzo et al., 2007; Ojeda et al., 2010). On the other hand, compounds with sulfhydryl groups are effective as free radical scavengers and as ACE inhibitors.

Fig. 1: Antioxidant activities of radish seeds: A) DPPH· radical scavenging capacity values; B) ORAC values; C) FRAP. Results expressed as µmol Trolox equivalents (TE)/g RSF. Tests were conducted in triplicate. Vertical lines represent standard deviations. Columns marked with different letters are significantly different (p<0.05).

Fig. 2: ACE inhibitory activities of antioxidant extracts of radish seeds. ACE inhibitory activity was measured from 1-ml extracts obtained from 2 mg of seed flour except for Yanzhi #2 (5D), which had a five-fold dilution. Tests were conducted in triplicate. Vertical lines represent standard deviations. Columns marked with different letters are significantly different (p<0.05).
inhibitors (TERRAS et al., 1992). So, it could not be overlooked that the ACE inhibitory activity of the extracts in this study was also associated with some other phytochemicals.

**Conclusions**

The results of this study confirmed that radish seeds are good sources of unsaturated fatty acids, tocopherols, lutein, and antioxidants. Radish cultivars may differ in seed composition and health properties; seed composition might be dependent on the geographical location. This study provides important information for developing value-added utilization of radish seeds or seed fractions such as oil and flour as nutraceuticals or functional food ingredients.

**Acknowledgments**

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**Conflict of interest**

Authors declare no conflict of interest.

**References**


KAYMAK, H.C., 2015: Profile of (n-9) and (n-7) Isomers of monounsaturated fatty acids of radish (Raphanus sativus L.) seeds. J. Am. Oil Chem. Soc. 92, 345-351. DOI: 10.1007/s11746-015-2600-0.


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