The Relations between Minor Components and Antioxidant Capacity of Five Fruits and Vegetables Seed Oils in China

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Abstract: The seed of five fruits and vegetables, which are often eaten by Chinese people, were selected as research objects to study the physicochemical properties, nutritional ingredients and antioxidant capacity of their seed oils. The fatty acid results indicated that the oleic acid was the main unsaturated fatty acid in almond oil and celery seed oil (content of 64.10\% and 62.96\%, respectively), and the wax gourd seed oil, watermelon seed oil and pumpkin seed oil were linoleic acid as the main unsaturated fatty acid (content of 72.45\%, 76.77\% and 47.35\%, respectively). Unsaturated fatty acids are mainly located at the sn-2 position of the triacylglycerol (TAG), whereas saturated fatty acids are mainly located at the sn-1, 3 positions for the five seed oils. The pumpkin seed oil had certain advantages in terms of phytosterols and squalene (3716 and 2732 mg/kg, respectively). The high content of polyphenol for celery seed oil exhibits higher medicinal value. Polyphenols, and brassicasterols were have significant correlation with antioxidant capacity ($p < 0.05, r = 0.890-0.998$). The significant differences in nutrient composition between these fruits and vegetables seed oils indicate their unique value as food.

Key words: seed oil, sn-2 fatty acid, nutrient composition, antioxidant capacity

1 Introduction

As the most important part of the plant breeding process, seeds often contain more nutrients than the root, leaf, and leaves of plants. Seed is the only oil-rich parts of most plants, so edible vegetable oils such as soybean oil, peanut oil, and olive oil are all extracted from the seed of plants.

The Chinese people often have the habit of using fruit and vegetable seeds as a snack, even regarding some seeds with medicinal value as health care products that regulate human functions. China, as a major producer and consumer country, has seen increasing demand for these foods in recent years. According to statistics, the China’s nut market reached 85 billion dollars in 2014, and it showed an increasing trend year by year. With the continuous improvement of people’s health awareness, many plant seeds with health-care functions have also been noticed. Therefore, researches on fruit and vegetable seeds that can be directly used as food has received more and more attention from scientists and food factories in recent years.

At present, research on oil in these seeds, which are nuts or health foods, is mostly concentrated on the analysis of nutrient components. Bertrand et al. researched the oil content, fatty acid composition and tocopherol content of some plant seeds (linseed, apricot, pear, fennel, peanut, apple, cotton, quince and chufa) as by-products of food processing industry\textsuperscript{1}). Oelschlägel et al. investigated the types and contents of phytosterols in different kinds of pumpkin seeds, watermelon, and cucumber seed oils\textsuperscript{2}). Seymen et al. reported the nutritional components of various pumpkin seeds, founding that the oil content was 33.04\% –46.97\% and rich in minerals such as potassium (2704.75–1033.63 ppm), phosphorus (3569.690–9108.835 ppm) and magnesium (1275.15–3938.16 ppm)\textsuperscript{3). Stevenson et al. re-
ported qualitative and quantitative analysis of tocopherols in various pumpkin seed oil, and γ-tocopherol and δ-tocopherol were the main components. Givianrad, et al. researched on the fatty acid composition and polyphenol content of almond oil, of which oleic acid and linoleic acid were the main fatty acids, and the polyphenol content was 45.3 mg/kg. Winter melon seed oil contained extremely rich polyunsaturated fatty acids, of which the proportion of linoleic acid was above 60%. The significant reduction of α-tocopherol content and linoleic acid content, elevated oleic acid content by microwave heating of sunflower oil was also observed in others paper.

However, there is no detailed report could be found in the literature about the nutrient and antioxidant capacity of oil in these seeds (pumpkin seeds, watermelon seeds, winter melon seeds, almonds, and celery seeds) that can be used as nuts or Chinese medicinal materials. Thus, it is of significant interest to measure free radical scavenging activity of these seed oils, and establish relationships between trace components and their antioxidant abilities. For this purpose, we aimed to explore the antioxidant properties of these seed oils based on the relationships of nutrients and free radical scavenging capacities. This will be beneficial to provide sufficient scientific basis for the future development and utilization of these fruit and vegetable seed resources.

2 Materials and Methods

2.1 Materials

The five fruit and vegetable seeds selected in experiment were purchased from their main producing areas in China. The seeds of wax gourd and celery were purchased from the vegetable farm in Shouguang City, Shandong Province; pumpkin seeds were purchased from the agricultural product processing plant in Bayannur City, Inner Mongolia Autonomous Region; watermelon seed were purchased from the agricultural product market in Bozhou City, Anhui Province, and the Almonds were purchased from the nut processing plant in Chengde City, Hebei province. All seeds were purchased in three batches to minimize changes in species or the effect of planting conditions on these materials.

Mixed standards of 37 fatty acid methyl esters (FAME) and tocopherols (purity >97%), 5α-cholestane, Trolox (water-soluble analog of vitamin E), porcine pancreatic lipase, and sodium cholate were all obtained from Sigma-Aldrich. Other inorganic reagents were purchased from Sinopharm Co., Ltd.

2.2 Methods

2.2.1 Seed oils extraction

About 300 g of fresh seeds were dehydrated with a freeze dryer, powdered by pulverizer, and then mixed with 1500 mL of petroleum ether at room temperature for 24 hours. Finally, the organic solvent was evaporated under reduced pressure in a rotary evaporator at 50°C to give the solvent-extracted seed oils.

2.2.2 Physicochemical properties

Acid Value (AV), Peroxide Value (PV), Saponification Value (SV) and Iodine Value (IV) were analyzed according to AOCS Official Method Cd 3d-63, AOCS Official Method Cd 8b-90, AOCS Official Method Cd 3-25 and AOCS Official Method Cd 1-25, respectively.

2.2.3 Fatty acid composition

The fatty acid composition of five seed oils were determined by gas chromatography after transesterification. The fatty acid methyl esters (FAMEs) were prepared by dissolved 100 mg seed oil in 10 mL of n-hexane, and reacting with 1 mL of a 1 M potassium hydroxide methanol solution at 40°C for 20 min. The supernatant was collected for GC analysis. The analysis was performed on an Agilent GC-2010 gas chromatograph equipped with a HP-88 capillary column (0.25 μm, 100 m x 0.25 mm, Agilent, USA) and a flame ionization detector (FID). The inlet temperature was 270°C and the detector temperature was 280°C. The initial column temperature was 130°C; it was then raised to 180°C at a rate of 7°C/min; then it was increased to 215°C at a rate of 3°C/min and maintained for 15 min; finally heated to 230°C at 4°C/min and hold for 3 min. Compared to the corresponding standard, it was confirmed that FAME retained time. The individual FAME is expressed as weight percent (area normalized).

2.2.4 Composition of sn-2 fatty acid

Composition of sn-2 fatty acid in triglycerides was determined according to the method described by Luddy et al. First, 2 mL of 1 mol/L Tris-HCl buffer (pH 8.0), 0.5 mL of 0.05% sodium bile solution, 0.2 mL of 2.2% CaCl₂ and 20 mg of pancreatic lipase were added to 100 mg of seed oil. The mixture was shaken at 40°C for 5 min, and then termination the reaction using 1 mL of 6 mol/L HCl. Finally, 2 mL of diethyl ether was added and centrifuged at 5000 r/min for 10 min. The upper phase was collected and separated using thin layer chromatography. The 2-monoylglycerol band was scraped off, extracted three times with anhydrous diethyl ether, and then removed organic solvent by nitrogen gas.

1.5 mL n-hexane was used to re-dissolve the dried 2-monoylglycerol and mixed with 0.5 mL 2 mol/L KOH-CH₃OH solution for methyl esterification. The prepared samples were measured by GC using the method in 2.2.3 to determine the sn-2 fatty acid composition.

2.2.5 Phytosterol in seed oils

Sterols and squalene were analyzed following the methods reported by Li et al. 100 mg seed oil was mixed with 0.2 mL internal standard (0.825 mg/mL 5α-cholestane) and 2 mL of a 2M potassium hydroxide methanol solution at 85°C for
1 hour. The mixture was cooled to room temperature and mixed with 5 mL of n-hexane and 2 mL of distilled water. The 1 μL of supernatant was detected by gas chromatography-mass spectrometry (5977B, Agilent, USA) with a DB-5MS capillary column (0.25 μm, 30 m × 0.25 mm, Agilent, USA). The injector temperature was 290°C, and the initial column temperature was set at 200°C, then raised to 300°C at 10°C/min and held at 300°C for 20 min. The carrier gas flow rate (helium gas) was 1.85 mL / min, the split ratio was 1:5, and the injection volume was 1 μL. In addition, the ion source and transmission line temperatures were 280°C and 250°C, respectively. The ionization mode is electron impact ion source (EI) and the mass range (m/z) is 50-700.

2.2.6 Composition of tocopherol

Tocopherols were analyzed using a high-performance liquid chromatography (HPLC) system (LC-20AT, Shimadzu, Japan) equipped with a fluorescence detector (RF-20A, Shimadzu, Japan). First, 500 mg of seed oil was weighed and diluted with 10 mL n-hexane. Then 20 μL of sample was injected into HPLC and separated by silica column (5 μm, 4.6 × 250 mm, Waters, USA). The mobile phase was n-hexane and isopropanol (99:1, v/v); the flow rate was 1 mL/min; the column temperature was set to 40°C; the excitation and emission wavelengths were 290 and 330 nm, respectively. The tocopherols of seed oil were identified and quantified by comparing the standards, and their contents were reported in mg/kg.

2.2.7 Total polyphenol

Refer to some paper, the total polyphenols were purified and measured using solid phase extraction (SPE) and Polin-Ciocalteu methods. About 1 g of seed oil was dissolved in 5 mL of n-hexane and then separated and purified using a Diol-SPE diethanol column, which was first activated with methanol and n-hexane. And then, the impurities of the seed oil in the column were eliminated with 5 mL of n-hexane and 5 mL of n-hexane-ethyl acetate (90/10, v/v). Finally, a methanol solution was added to the SPE column to collect the polyphenols in seed oils. 5 mL of the methanol collection and 2 mL of 25% folin phenol was added to the 10 mL volumetric flask and mixed for 1 min. Then the mixture was added 1 mL of 10% Na₂CO₃ solution, and diluted the final volume to 10 mL with water. The absorbance was measured at 760 nm after 1.5 hours reaction in darkness. Gallic acid and pure water were used as reference standards and blank samples, respectively. The result is expressed as mg GAE (gallic acid equivalent/kg oil.

2.2.8 Free radical scavenging capacity

5 mL of methanol was mixed with 4 g of seed oil and shaken vigorously for 5 min in darkness. And then the mixture was centrifuged at 5000 rpm for 10 min using a high speed centrifuge. The upper layer solution was transferred into 25 mL brown volumetric flask, and the above extraction process repeated 4 times, using 5 mL of methanol each time. Finally, all methanol extracts were collected, sealed with nitrogen and stored at −20°C.

Using three methods (DPPH, FRAP (iron potential for reducing antioxidants), and ABTS) for determining the scavenging ability of five seed oils. The Trolox (water-soluble analogue of vitamin E) was used as a reference standard to construct a standard curve for quantitative analysis of the antioxidant capacity of seed oil, and the results were expressed as μmol of Trolox equivalents per 100 g of seed oil, i.e. μmol TE/100 g oil.

ABTS assay: In brief, 0.44 mL of potassium persulfate solution (2.45 mmol/L) was added to 25 mL ABTS reagent (7 mmol/L) to obtain the ABTS stock solution. The ABTS working solution was prepared by placing the stock solution in the dark for 12 hours and diluting with methanol until an absorbance of 0.700 ± 0.020 was reached at 734 nm. Finally, 0.1 mL of methanol extract of seed oil and 3 mL ABTS working solution were reacted in the dark at room temperature for 20 min. The absorbance was measured at 734 nm.

DPPH assay: Some optimizations have been made according to the reported methods. Briefly, 2 mL of methanol sample extract was reacted with 2 mL of DPPH reagent (0.5 mmol/L) in darkness at room temperature for 1 hour.

FRAP assay: The FRAP capacity of the five kinds of seed oil was evaluated according to the procedure described by Aleksandra et al. with some modifications. First, HCl solution (40 mmol/L) and TPTZ reagent were mixed to prepare a 10 mmol/L TPTZ stock solution. Then, the FRAP working solution was prepared by mixing 25 mL of 0.1 mol/L acetate buffer, 2.5 mL of TPTZ solution and 2.5 mL of 20 mmol/L FeCl₃ solution at 37°C. Finally, 0.2 mL of the methanol extract and 4 mL of FRAP working solution were reacted for 10 min at 37°C. Blank and samples were measured at 593 nm.

2.2.9 Data Analysis

All samples were analyzed in triplicate and the results were reported as mean ± standard deviation. Statistical analysis was performed using Origin 2017 (Origin lab, Northampton, MA, USA) and SPSS 19.0 (IBM, Armonk, NY, USA). Data were compared by one-way analysis of variance (ANOVA), and significant differences between the variables of the test seed oils were assessed by Turkey’s-b tests at p < 0.05. The multiple linear regression (MLR) analysis using a stepwise method was used to evaluate the correlations between the obtained results.

3 Results and Discussion

3.1 Physicochemical properties

Table 1 shows the physicochemical properties of the five seed oils, including density, refractive index (RI), acid value (AV), peroxide value (PV), iodine value (IV), and sa-
ponification value (SV). The AV of wax gourd seed oil (4.96 mg KOH/g oil) and watermelon seed oil (4.46 mg KOH/g oil) was higher than 2 mg/g as European Union stated for edible product standard, which means that the two seed oils should be further refined. The PV of the five seed oils were all less than 19.7 meq O2/kg oil. And SV is generally inversely proportional to the average relative molecular mass of the TAG in the oil. The higher SV of wax gourd seed oil and pumpkin seed oil may indicate the lower relative molecular weight of TAGs. In addition, the IV of the five seed oils were ranged from 81.5 g iodine/100 g oil to 121.1 g iodine/100 g oil, which watermelon seed oil had the highest IV, followed by pumpkin seed oil, and almond oil had the lowest. IV is usually proportional to the amount of unsaturated fatty acids in the lipid. The poor IV value of almond oil represented a lower degree of unsaturation, and higher IV observed in watermelon seed oil and pumpkin seed oil indicated that they were rich in unsaturated fatty acids.

### 3.2 Fatty acid composition

The percentage of fatty acids was also showed in Table 2. It can be seen that linoleic acid (C18:2 n6), oleic acid (C18:1), palmitic acid (C16:0) and stearic acid (C18:0) were the four kinds of primary fatty acids in the five seed oils, which accounted for 86–99% of the total component of all the samples. These seed oils were rich in unsaturated fatty acids, and the contents ranged from 81.87% to 90.73%. Compared with vegetable oils commonly eaten by Chinese people, the unsaturated fatty acids of these seed oils were characterized by concentrated species and high content.

Linoleic acid was the highest content of fatty acids in wax gourd seed oil, watermelon seed oil and pumpkin seed oil. The former two were one of the best sources of linoleic acid, and the proportions accounted for 72.45% and 76.77% of the total fatty acids contents, respectively. The contents of oleic acid and linoleic acid in pumpkin seed oil were 34.24% and 47.35%, respectively. Oleic acid, the main fatty acid in celery seed oil and almond oil, accounted for 64.10% and 62.96% respectively. These results are consistent with the conclusions of many researchers. The fatty acid characteristics of the tested seed oils indicated that they have unique nutritional value in terms of lipid-lowering and immunity enhancement.

In addition, a few low-abundance fatty acids were also found in our study. The palmiteoleic acid (0.05%–0.44%) and heptadecanoic acid (0.05%–0.09%) were found in other four seed oils except celery seed oil. It is worth mentioning that all these samples contain nervonic acid, which is an important nutrient that can repair human nerve cells and increase the vitality of brain cells, and it also has a certain effect in improving immunity and treating AIDS.

### 3.3 Sn-2 fatty acid

The relative percentage of each fatty acid at the sn-2 position was calculated by formula \((\text{sn-2 fatty acid} / 100 / \text{total fatty acid})\), and the results were presented in Table 2, which was better to understand the stereochemical structure of triglycerides. The saturated fatty acids at the sn-2 position were much lower than unsaturated fatty acid. The C16:0 and C18:0 were predominantly located at the sn-1, 3 positions in these five seed oils. The measurement results of sn-2 fatty acid showed different distributions from the fatty acid. Linoleic acid was the main sn-2 fatty acid in wax gourd, pumpkin, celery and almond seed oil, account for 42.11%, 51.01%, 68.68% and 48.76%, respectively. While the oleic acid percentage at the sn-2 position was 62.23% in watermelon seed oil.

Some researches indicate that, due to such reasons as steric hindrance, fatty acids in the sn-2 position are more difficult to be oxidized. The above mentioned results showed that the C18:1 and C18:2 were mainly located at the sn-2 position in five seed oils. This means that these seed oils having unsaturated fatty acids linked at the sn-2 position of glycerol are more stable than those vegetable oils. With the further development of the research, there has been increasing interest in the location of polyunsaturated fatty acids (PUFAs) in triglycerides, as several studies have supported that these PUFAs at the sn-2 site are more readily absorbed than triglycerides with random
distribution of PUFAs. Therefore, the determination of the distribution of fatty acids in triglycerides is beneficial for the accurate evaluation of the nutritional value of fatty acids in vegetable oils.

3.4 Phytosterol and squalene
Phytosterols are cholesterol-like molecules found in high concentrations of vegetable oils, synthesized from squa- lene, and help inhibit intestinal cholesterol absorption, including cyclic cholesterol cycling. Four phytosterols (brassicasterol, campesterol, stigmasterol, and β-sitosterol) and squalene were measured and compared in the five seed oils and the results were shown in Table 3.

| Wax gourd | Pumpkin | Watermelon | Celery | Almond |
|-----------|---------|------------|--------|--------|
| C6:0      | 0.13 ± 0.02b | -a         | -a     | -a     | -a     |
| C8:0      | -a       | 0.04 ± 0.01a | 0.11 ± 0.01a | 0.33 ± 0.06a | 0.09 ± 0.00c |
| C10:0     | -a       | 0.06 ± 0.00b | -a     | 0.11 ± 0.03c | -a     |
| C11:0     | -a       | -a          | -a     | 4.19 ± 0.67a | -a     |
| C12:0     | -a       | -a          | -a     | 0.05 ± 0.01b | -a     |
| C14:0     | -a       | 0.12 ± 0.01a | 0.06 ± 0.00b | 0.07 ± 0.02c | -a     |
| C14:1     | 0.04 ± 0.00b | -a          | 0.06 ± 0.02c | -a     | 0.13 ± 0.00d |
| C15:0     | -a       | -a          | -a     | 0.06 ± 0.00b | -a     |
| C16:0     | 11.42 ± 1.18c | 12.20 ± 0.95c | 9.28 ± 0.61b | 5.27 ± 1.21c | 5.23 ± 0.03a |
| C16:1     | 0.05 ± 0.01a | 0.13 ± 0.02c | 0.07 ± 0.00b | -a     | 0.44 ± 0.04d |
| C17:0     | 0.07 ± 0.01c | 0.08 ± 0.02d | 0.05 ± 0.00b | -a     | 0.09 ± 0.00c |
| C17:1     | 0.12 ± 0.03c | -a          | 0.03 ± 0.01b | -a     | -a     |
| C18:0     | 6.40 ± 0.57d | 5.25 ± 0.77c | 3.60 ± 0.10b | 1.01 ± 0.01a | 3.63 ± 0.03b |
| C18:1n9   | 8.54 ± 0.46d | 34.24 ± 0.63c | 8.54 ± 0.60a | 64.10 ± 3.20b | 62.96 ± 4.92c |
| C18:2n6   | 72.45 ± 3.22d | 47.35 ± 2.03b | 76.77 ± 0.39a | 16.42 ± 0.09c | 62.70 ± 0.51b |
| C18:3n-3  | 0.47 ± 0.03d | 0.06 ± 0.00b | 0.30 ± 0.09c | 0.48 ± 0.03b | 0.38 ± 0.01b |
| C20:0     | 0.11 ± 0.01b | 0.25 ± 0.01d | 0.04 ± 0.00b | 4.90 ± 0.24d | 0.23 ± 0.21c |
| C20:2     | -a         | -a          | 0.19 ± 0.04b | 1.07 ± 0.14d | 0.05 ± 0.01b |
| C20:3n6   | 0.09 ± 0.01b | -a          | -a     | -a     | -a     |
| C22:2     | -a         | -a          | 0.22 ± 0.01b | -a     | -a     |
| C24:1n9   | 0.11 ± 0.01c | 0.08 ± 0.02b | 0.30 ± 0.04a | 0.14 ± 0.01b | 0.07 ± 0.00a |
| C24:0     | -a         | -a          | 0.38 ± 0.00b | 1.80 ± 0.21a | -a     |
| SFA       | 18.13c     | 18.00c      | 13.52c  | 17.79c  | 9.27c   |
| UFA       | 81.87d     | 82.00d      | 86.48d  | 82.21d  | 90.73b  |
| MUFA      | 8.86c      | 34.45b      | 9.00c   | 64.24c  | 63.60c  |
| PUFA      | 73.01d     | 47.55c      | 77.48c  | 17.97c  | 27.13b  |

Sn-2 Fatty acid compositions (%)

| Wax gourd | Pumpkin | Watermelon | Celery | Almond |
|-----------|---------|------------|--------|--------|
| C16:0     | 6.71d   | 6.09b      | 14.97d  | 16.95c  | 4.53c   |
| C18:0     | 6.82c   | -a         | 35.46d  | 27.06d  | 4.22b   |
| C18:1     | 16.78c  | 23.88b     | 62.23d  | 32.25b  | 31.44c  |
| C18:2     | 42.11b  | 51.01d     | 32.40c  | 68.68c  | 48.76d  |

SFA, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

“-” Not detected. Values in the same row with different letters are significant difference at p < 0.05.
smaller amounts in other vegetable oils to 2732 mg/kg. In pumpkin seed oil, the contents of squa-
scelerol was found in celery seed oil, and the contents ranged from 113.7 mg/kg detected in pumpkin seed oil, watermelon seed oil and almond oil, the other four seed oils contain high content of 

β-bond of sitostanol formed after the opening of the alpha double 

bond of sitostanol formed after the opening of the alpha double 

The tocopherol contents in the five seed oils were shown in Table 3. Large differences in tocopherol compositions and total contents of the five seed oils were found, for instance, wax gourd seed oil showed the highest (717.4 mg/kg), while the celery seed oil had the lowest, only 133.4 mg/kg. α-tocopherol and δ-tocopherol were found at lower 

5 52.4 and 90.6 mg/kg, respectively) in these seed oils. Wax gourd seed oil showed the highest γ-tocopherol contents (612.9 mg/kg), followed by water-

melen seed oil and celery seed oil (552.4 and 90.6 mg/kg, respectively). Schwartz et al. 25 testified that α-and γ-tocopherols were the major tocopherols in vegetable oils. The main tocopherol in pumpkin seed oil and almond oil was β-tocopherol (241.6 mg/kg and 317.1 mg/kg, respectively), which was 10 to 50 times higher than common vegetable oils40. In many literatures, α-tocopherol was report-

ed as the main ingredient in almond oil. But Chinese literature 33 shows that β-tocopherol was the main tocopherols in almond oil. Therefore, whether almonds produced in China have unique tocopherol composition remains to be further studied.

### 3.6 Polyphenols content

Vegetable oils contain polyphenol compounds, which play an important role in anti-inflammatory, antibacterial

### Table 3 Minor components of five seed oils (mg/kg).

| Phytoestrol | Wax gourd | Pumpkin | Watermelon | Almond | Celery |
|-------------|-----------|---------|------------|--------|--------|
| Brassicasterol | -a       | -a      | -a         | -a     | 1597 ± 7.0b |
| Campesterol | 2256 ± 53.2c | 2276 ± 49.8c | 2083 ± 38.1b | 2091 ± 41.1b | 1342 ± 30.8a |
| Stigmasterol | 980.8 ± 17.9c | 747.2 ± 18.1i | 368.4 ± 2.7a | -a     | 424.7 ± 1.3c |
| β-Sitosterol | -a       | 693.1 ± 7.4e | -a         | -a     | 639.6 ± 5.7b |
| Total       | 3237d    | 3716d   | 2452b      | 2091a   | 4003c   |
| Squalene    | -a       | 2732 ± 22.7d | 113.7 ± 4.3b | -a     | 569.3 ± 6.9c |

Tocopherol

| α-tocopherol | 90.6 ± 6.3d | 4.7 ± 1.4e | 14.1 ± 2.1b | 14.5 ± 2.6b | 27.1 ± 1.6c |
| β-tocopherol | -a         | 241.6 ± 3.7b | -a         | 317.1 ± 14.3c | -a |
| γ-tocopherol | 612.9 ± 13.5d | -a    | 552.4 ± 9.6c | -a     | 90.6 ± 3.4b |
| δ-tocopherol | 13.9 ± 2.2d | 6.8 ± 1.1e | 24.1 ± 2.8c | 17.8 ± 0.8d | 15.7 ± 1.4c |
| Total       | 717.4a     | 253.1b   | 590.6d     | 349.4c   | 133.4a   |

Polyphenols (mg GAE/kg oil)

| Wax gourd | Pumpkin | Watermelon | Almond | Celery |
|-----------|---------|------------|--------|--------|
| 56.4 ± 3.2b | 75.0 ± 4.1c | 187.3 ± 9.4d | 36.0 ± 1.54a | 1250 ± 67.3c |

< 0.05.

mg GAE/kg oil, mg gallic acid equivalent per 100 g seed oil.

“, “, Not detected. Values in the same row with different letters are significant difference at p < 0.05.
and anti-oxidation. The contents of polyphenols in the five seed oils were shown in Table 3. Total polyphenol contents presented high variation among different seed oils, which ranged from 36.0 mg GAE/kg oil to 1250 mg GAE/kg oil. As exhibited in Table 3, celery seed oil showed significantly higher contents of the total polyphenol (1250 mg GAE/kg oil), followed by watermelon seed oil (187.3 mg GAE/kg oil). It is worth mentioning that celery seed oil contains extremely rich polyphenols, which were 6.7 to 34 times more than other four seed oils. Besides, although the polyphenol amount of the watermelon seed oil was lower than celery seed oil, however, it still relatively high amounts than most vegetable oils. The results of this study demonstrated that celery seed oil shows obvious advantage in the contents of polyphenols. Thus, it is necessary to further study the physiological effects of polyphenols in celery seed oil.

3.7 Free radical scavenging capacity

The three methods (DPPH, ABTS and FRAP) were used to evaluate the free radical scavenging ability of the polar parts of the five seed oils. The results of scavenging free radicals from different seed oils were shown in Fig. 1.

It can be seen from the measurement results that the celery oil has good performance in the three free radical scavenging capacity tests, and its scavenging capacity was far greater than the other four seed oils. The ABTS free radical scavenging capacity of celery seed oil (157.9 μmol TE/100 g oil) was close to that of canola oil, and the wax gourd and almond seed oils (61.3 μmol TE/100 g oil and 57.5 μmol TE/100 g oil, respectively) were similar to the sunflower seed oil (19.6 μmol TE/100 g oil). The DPPH free radical scavenging activity of celery seed oil was 346.6 μmol TE/100 g oil. The celery seed oil showed greater DPPH free radical scavenging activity than sesame oil (245 μmol TE/100 g oil) and safflower oil (255 μmol TE/100 g oil), which were considered to have a high ability to scavenge free radicals. The DPPH free radical scavenging capacity of watermelon and pumpkin seed oils (43.1 μmol TE/100 g oil and 66.2 μmol TE/100 g oil, respectively) were higher than common vegetable oil such as soybean oil and the wax gourd and almond seed oils (142.9 μmol TE/100 g oil and 138.4 μmol TE/100 g oil, respectively) were similar to the rapeseed oil. In experiments that eliminated FRAP free radicals, the ability of celery oil (192.3 μmol TE/100 g oil) was still the highest, and it was equivalent to the olive oil, but the other four seed oils were poor. The results indicated that the celery oil has a good free radical scavenging capacity, which may be related to its high content of polyphenols.

3.8 Correlation between phytochemistry and free radical scavenging capacity

The correlation between the nutrient composition and antioxidant capacity of the test seed oil was analyzed by calculating the correlation coefficient, which was generally used to describe the degree of association between two consecutive variables. As can be seen from Table 4, there was a significant correlation between polyphenols and antioxidant ability indicated by ABTS, FRAP and DPPH (r = 0.971, 0.986 and 0.890, respectively). In addition, good correlations were observed between brassicasterol and free radical scavenging ability of the tested seed oils as well. The phenolic hydroxyl groups of polyphenols combine with free radicals in seed oil to turn them into more stable materials, giving the seed oil excellent oxidative stability. Thus, celery seed oil exhibited strong ability in the free radical scavenging experiments. And the levels of these nutrients in almond oils were not abundant, so the almond seed oil showed the poor ABTS and FRAP free radical scavenging capacity. The above results indicated that polyphenols and brassicasterol have significant correlation with the antioxidant capacity of seed oil. Whereas, there may be other non-polar compounds in these seed oils affecting on antioxidant activity that was not tested or have synergistic effects between nutrients, because synergistic effects and antagonistic effects are prevalent among nutrients.

3.9 Multiple Linear Regressions (MLR)

Based on the results of previous experiments, a stepwise MRL was used to determine the factors associated with the free radical scavenging activities of the seed oil, and MRL analysis was performed on all tested secondary com-
Fig. 2 shows all predicted values were within 95% of the confidence interval, so there were high correlation in all predicted and observed values of the proposed model, which had statistically significant $p \leq 0.05$.

Table 5 shows multiple linear regression analysis between antioxidant capacity and nutrient composition. The results showed that polyphenols and brassicasterol were the main independent variables for scavenging free radical tests. It was basically consistent with the results of correlation analysis. Polyphenols containing hydroxyl groups were significantly correlated with the results in ABTS and FRAP experiments, the partial correlation coefficient of the predicted equations were 0.971 and 0.986, respectively. And brassicasterol was the correlative significant variable for DPPH and FRAP, the partial correlation coefficient of the predicted equations were 0.931 and 0.957, respectively.

The relationship between phytochemical and free radical scavenging capacity has been reported in the literature. The reaction principles of ABTS and FRAP were the same as that of polyphenol methods, except for the different pH values in the reaction environment. They evaluate the antioxidant ability of lipid to transfer one electron to free radical. In contrast, DPPH is based on the hydrogen atom transfer, which removed a hydrogen atom from the antioxidants to form a stable radical group. Therefore, this is why the MLR equations had different independent variables. These results indicate that the main contribution factors of different scavenging free radical assay are different, and multiple assays are needed to comprehensively evaluate the antioxidant capacity of certain oils.
Conflicts of interest
The authors declare no competing financial interest.

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