Comparative Analysis of Mineral Elements and Essential Amino Acids Compositions in *Juglans sigillata* and *J. regia* Walnuts Kernels

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Abstract

Walnut kernel is famous for its high nutritional and economic values. The kernel is usually considered to be a good source of minerals and essential amino acids. In this paper, mineral elements (calcium, magnesium, iron, manganese, copper, and zinc) and essential amino acids (phenylalanine, valine, threonine, isoleucine, leucine, methionine, and lysine) compositions of kernels from 11 kinds of walnuts (*Juglans sigillata*) and 17 kinds of walnuts (*Juglans regia*) originated from China were determined by ICP-MS and HPLC, respectively. The order of nutritive mineral elements depending on their content (mg/100g) of samples was Mg > Ca > Zn > Mn > Fe > Cu in *J. regia*, while the order in *J. sigillata* was Mg > Ca > Mn > Fe > Zn > Cu. For essential amino acids, the order depending on the content (mg/g) of the essential amino acids in *J. regia* samples was leucine > isoleucine > valine > phenylalanine > lysine > threonine > histidine, while the order in *J. sigillata* was leucine > isoleucine > lysine > phenylalanine > valine > threonine > methionine. The kernels of walnuts (*J. regia* and *J. regia*) are good sources of health foods and dietary supplements. 'Y029' in *Juglans sigillata* and 'XJ004' in *Juglans regia* provided the best profiles of mineral elements and essential amino acids in comparison to others.

Keywords: essential amino acid, HPLC, ICP-MS system, mineral element, walnut kernel

Introduction

Walnuts (*Juglans regia* and *Juglans sigillata*) are widely distributed in China. There is a variety of walnuts differing in varieties, productivity, physical and chemical features of their kernel. Some of them have been evaluated as promising and may serve as germplasm sources for breeding (Warmund *et al.*, 2009; Martínez *et al.*, 2010; Qin *et al.*, 2012; Albuquerque *et al.*, 2013). As we all know, the kernel of walnut as food has remarkable nutrients such as quinones, oils, tannins, essential fatty acids, albumin, mucilage, mineral matter, amino acids, and so on. Thus, walnut is also considered to be a good source of minerals and essential amino acids. In generally, significant quantities of magnesium (Mg), calcium (Ca), phosphorus (P), potassium (K), and iron (Fe) are present in the kernels. Researchers are conducted to study on the contents of nutrition elements in cultivated walnuts and their impact on human health. It was found that an adequate intake of walnut kernel could be beneficial to lowering blood cholesterol and improving arterial function (Sabate *et al.*, 1993; Savage, 2001; Nash and Westpfal, 2005; Patel, 2005).

Moreover, the amino acids regarded as essential nutrients for humans are phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, lysine, and histidine (Young, 1994). Importantly, cysteine (or sulphur-containing amino acids), tyrosine (or aromatic amino acids), and arginine are essential for infants and growing children (Imura and Okada, 1998; Joint, 2007). The essential amino acids play a key role in the development of body since they are not synthesized by people. While the amino acids including arginine, cysteine, glycine, glutamine, histidine, proline, serine, and tyrosine are considered conditionally essential (Reeds, 2000; Fürst and Stehle, 2004). For example, tyrosine is essential for the patients with the disease of phenylketonuria (PKU). In the present study, Wu *et al.* (2007) reported that there were 16 kinds of amino acids in the 4 kinds of walnut kernels besides of glutamic acid. The amino acids included 7 kinds of essential amino acids, in which the content of leucine was the highest. In comparison, the content of amino acids from Pao-walnut kernels was significantly (p<0.01) lower than those from other groups. In addition, amino acids were also determined in other varieties of walnut kernels (Yanbin, 2002; Qinghu *et al.*, 2003).

Alternatively, mineral elements have been identified as valuable compositions in kernels of walnuts. The previous research found that there were 5 main kinds of mineral elements (K, P, Mg, Ca, and Mn) in kernels of walnut cultivars 'Franquette' and 'Hartley' originated from France and California, respectively, in which the contents of K, P, and Mn were significant differences according to the factors like the varieties of walnuts, growth conditions, and climate (Lavedrine *et al.*, 2000). The mineral elements in 5 kinds of walnut genotypes (‘Sebin-Type-I’, ‘Kürcügöz’, ‘Karabodur’, ‘Tozam’, and ‘Güvenli’) cultivated in Turkey were also determined by Caglarirmak (2003). It was found that the mean contents of mineral elements were 316.0, 270.0, 85.0, 90.0, 2.0, 2.5, 2.9, and 1.0 mg/100g for P, K, Ca, Mg, Zn,
Mn, Cu, and Fe, respectively. Chemical analysis revealed the composition to be 13.77% protein, 62.84% fat, 18.67% total carbohydrates, 1.81% ash, and 2.98% moisture (Caglarirmak, 2003). Recently, selenium (Se) was also found in 15 kinds of walnut kernels (J. regia) grown in Erzincan and the contents of Se were ranged between 7.25 and 57.67 ng g⁻¹ (Ozrenk et al., 2012).

Furthermore, by the analysis of chemical compositions in walnut kernels from New Zealand, USA (‘Tehama’ and ‘Vina’), and Europe (‘Estherhazy’, ‘G120’, ‘G139’), the results showed that the contents of proteins were between 13.6% and 18.1%, and the contents of amino acids were similar among cultivars (Savage, 2001). In addition to proteins, amino acids, and minerals, tocotrienol and fiber were also found in kernels of walnuts (Sze-Tao and Sathe, 2000; Ozrenk et al., 2012). In this paper, the aim of our study was to determine the contents of mineral elements and essential amino acids in kernels from 11 kinds of walnuts J. sigillata and 17 kinds of walnuts J. regia collected in China.

Materials and methods

Materials

The kernels from 11 kinds of walnuts J. sigillata (‘Y021’, ‘Y026’, ‘Y029’, ‘Y041’, ‘Y042’, ‘Y075’, ‘Y082’, ‘Y096’, ‘Y098’, ’Y099’, and ’Y101’) and 17 kinds of walnuts J. regia (‘X1004’, ‘X1005’, ‘X1008’, ‘X1012’, ‘X1017’, ‘X1018’, ‘X1019’, ‘X1020’, ‘X1029’, ‘SX05’, ‘SX13’, ‘SS004’, ‘SAK002’, ‘SHZ002’, ‘XI’, ‘X9’, and ‘SS002’) were obtained in Shaanxi (lat. 34°12’~34°20’N, long. 108°~108°7’E) and Yunnan (lat. 27°08’~27°15’N, long. 101°31’~102°11’E) provinces in China, respectively. The climate is characterized by the annual mean rainfall of 635.1 mm and mean temperature of 12.9 °C for Shaanxi province, and 1 300 mm and 16 °C for Yunnan province. Kernels were kept in glass jars until analyses at refrigerator. In all stages of experiments, dry and mature kernels were used. Azotic acid (65%, Fluka), oxygenated water (33%), ethanol, triethylamine, phenyl etherlysate (PTIC, 99.9%), and ultrapure water were used as received.

Methods

The digestion microwave system (Milestone Inc., USA) was used for the mineralization of solid sample. 0.5 g samples, 6 mL 65% nitric acid, and 2 ml 33% hydroperoxide were introduced in Teflon recipients. Then, the oven temperature was increased to 180 °C at 5 °C min⁻¹ and held for 20 min. After cooling to room temperature, the liquid samples were transferred into the marked glass balloons and the total volume of 50 ml was obtained with ultrapure water, and ultrapure water and ultrapure purity commercial acids were used to prepare all reagents, standards and walnut kernel samples. After digestion treatment, samples were filtrated through whatman No 42 (Özcan et al., 2010). The filtrates collected for the mineral elements determination were analyzed by ICP-MS (Perkin-Elmer Elan 9000). The ICP torch was fusse type and ceramic alumina torch injector. Nebulizer was tip cross flow and the flow of gas is 0.93 L min⁻¹. Additionally, the parameters of the spray chamber were sweeps/reading 20 s, reading/ replicate 2, and number of replicates 5.

The compositions of amino acids were determined by amino acid analyzer with a Pico-Tag column. Accurately weighed sample (0.1 g) was hydrolyzed with 600 µL HCl (6 M) at 110 °C for 18 h under nitrogen. After hydrolysis, the hydrolysat was dried, mixed with ethanol / triethylamine / water solution (2:2:1, v/v/v) and dried again. Then the dried sample was derivatized with an ethanol/triethylamine/water /PTIC (7:1:1:1, v/v/v/v) solution at 25 °C for 20 min under nitrogen and then dried. Finally, the sample was dissolved in 50 µL of 5 mM sodium acetate buffer (pH 7.6, 40 °C) containing 6% (v/v) acetonitrile and analyzed by HPLC. Norleucine was used as an internal standard to calculate percent recovery of amino acids, while tryptophan was determined by the method of colorimetric (No. 3) (Spies and Chambers, 1948). The contents of amino acids were reported as grams of amino acid per 100 g of protein.

Statistical analyses

All the data were analyzed using the SPSS 16.0 statistical software for analysis of variance using ANOVA and Duncan’s least significant difference (LSD at p < 0.05) for statistical significance. This research was performed by 3 duplicates with a replicate, and data were reported as the mean ± standard error of the mean.

Results and discussion

Mineral elements

As known, the kernels of walnut have a remarkable amount of mineral nutrition that is beneficial to body health among common foodstuffs. Actually, the kernels contain many kinds of minerals, in which Mg and Ca were the major minerals. Tab. 1 shows the results of all the mineral compositions in kernels from 11 kinds of walnuts J. sigillata and 17 kinds of walnuts J. regia. Ca, Mg, Fe, Mn, and Zn were determined by atomic absorption spectrometry. From the Tab. 1, Mg and Ca showed the higher contents than others in the determined samples. The order depending on the contents of elements (mg/100g) in J. regia samples was Mg> Ca> Zn> Mn> Fe> Cu, while the order in J. sigillata was Mg> Ca> Mn> Fe> Zn> Cu. Recent studies showed that osteoporosis, hypertension, coronary heart disease, congestive heart failure, arrhythmia, diabetes mellitus, asthma, migraine headaches, and pre-eclampsia may be associated with the deficiency of Mg (Touyz 2004). In Tab. 1, the contents of Mg varied between 401.47 (‘X1’) and 749.27 mg/100g (‘SS002’) in kernels of J. regia, 538.40 (‘Y075’) and 771.93 mg/100g (‘Y096’) in kernels of J. sigillata. The mean values of Mg were 579.18 mg/100g in J. regia and 647.57 mg/100g in J. sigillata. The ratio of the mean contents of Mg in J. sigillata to J. regia was I.1.1.

It was followed by Ca, which is an essential nutrient and a vital electrolyte for human body (Bryant et al., 1999). In addition, it is well known that Ca plays an important role in the development of bone and teeth. The determination showed that the contents of Ca were changed between 155.10 (‘Y096’) and 482.03 mg/100g (‘Y101’), and the mean value was 288.42 mg/100g in J. sigillata. The contents of Ca varied between 253.90 (‘XJ004’) to 504.07 mg/100g (‘SS002’) in J. regia, and the recorded mean value was 352.18 mg/100g. The ratio of the mean contents of Ca in J. regia to J. sigillata was 1.22.
Zn is one of the most abundant nutritionally essential elements in the human body. It is found 85% of the whole Zn in muscle and bone, 11% in the skin and the liver, and the remaining in all the other tissues (Tapiero and Tew, 2003). In this paper, Zn was limited between 6.84 (‘X1’) and 11.42 mg/100g in ‘Y098’ from J. sigillata. The mean values of Zn were 8.17 mg/100g in J. regia and 11.42 mg/100g in J. sigillata. The ratio of the mean contents of Fe in J. sigillata to J. regia was 1.40.

Cu is a kind of dynamic element and associated with anti-infectious, antiviral, anti-inflammatory. In addition, Cu is an essential trace element in living organisms, functioning as a cofactor for many enzymes (Tapiero et al., 2003; Fraga, 2005). The kernels of walnuts are rich sources of Cu, and the variation of Cu was limited between 1.37 (‘SS004’) and 5.30 mg/100g (‘XJ008’) in J. regia, 1.46 (‘Y099’) and 6.91 mg/100g (‘Y098’) in J. sigillata. The mean contents of Cu were 3.72 mg/100g in J. regia and 4.93 mg/100g in J. sigillata. The ratio of the mean contents of Cu in J. sigillata to J. regia was 1.33.

As a result, the order depending on the contents of elements in J. regia samples was Mg> Ca> Zn> Mn> Fe> Cu, while the order in J. sigillata was Mg> Ca> Mn> Fe> Zn> Cu, which was similar with the order of mineral
contents (Mg > Ca > Mn > Zn > Fe > Cu) in walnut kernel (J. regia L.) from USDA National Nutrient Database (Tab. 2) (USDA, 2013).

Compositions of essential amino acids

The compositions of essential amino acids are summarized in Tab. 3. The essential amino acids including phenylalanine, valine, threonine, isoleucine, leucine, methionine, and lysine were detected by HPLC. As can be seen in Tab. 3, the highest content of essential amino acid was leucine in both J. sigillata and J. regia, while the lowest one was methionine. The order depending on the contents of the essential amino acids (mg/g) in J. regia kernels was methionine > isoleucine > valine > phenylalanine > lysine > threonine > leucine. In J. sigillata, the order was in the same way except that leucine was replaced by valine.

The mean content of threonine in J. sigillata was 16.24 mg/g. In comparison, the highest one was 18.32 mg/g in kernels of 'Y075', which made up to 10.26% of total threonine in J. sigillata. While the highest content of threonine in kernels of 'XJ004' was 17.72 mg/g, which reached 7.51% of total threonine in J. regia and the mean value of threonine in J. regia was 13.87 mg/g. From the analyzed results, the ratio of total contents of threonine in J. sigillata to J. regia was 1.17.

Tab. 2. Mineral contents of walnut (J. regia) kernel (mg/100g, dry weight) from USDA National Nutrient Database and walnuts (J. regia and J. sigillata)

Tab. 3. Compositions of essential amino acids (mg/g, dry weight) in kernels from 11 kinds of walnuts (J. sigillata) and 17 kinds of walnuts (J. rega)

The same letter indicated that the values were not significantly different at P < 0.05, according to Duncan's multiple range test.
The following one is valine, which can accelerate the wound healing, treatment of liver failure, raise blood sugar levels, and increase the content of growth hormone. In Tab. 3, the highest content of valine was 23.47 mg/g for 'Y029', which reached 10.93% of the total aine in the kernels of 'Y029' from J. sigillata, and the mean value of valine was 19.53 mg/g for J. sigillata. At the same time, the highest content (23.93 mg/g, 'XJ004') of valine in J. regia was very similar to that in J. sigillata. While the highest content was just up to 8.94% of the total content of valine and the mean value was 15.75 mg/g for J. regia. The ratio of the mean values of valine in J. sigillata and J. regia was 1.24.

Moreover, Tab. 3 shows that the lowest content of essential amino acids was methionine. The mean content of methionine was just 6.58 mg/g in J. sigillata, while the highest content of methionine was 7.73 mg/g in 'Y021'. Meanwhile, the highest content of methionine was 7.47 mg/g (‘XJ004’) in J. regia and the mean value was 6.28 mg/g, which was very similar to the mean content of methionine in J. sigillata. Cysteine and methionine are 2 kinds of sulfur-containing proteinogenic amino acids. Loss of methionine has been linked to senile greying of hair and leads to the accumulation of hydrogen peroxide in hair follicles, which reduces the effectiveness of tyrosinase to loss color of hair (Wood et al., 2009).

What's more, isoleucine was the second highest content of essential amino acids in both J. sigillata and J. regia. The highest values of isoleucine were 33.18 mg/g in 'Y029' from J. sigillata and 47.53 mg/g in 'X9' from J. regia. The mean contents of isoleucine were 30.53 mg/g in J. sigillata and 30.24 mg/g in J. regia. A research showed that methionine supplement in the diet specifically increased mitochondrial ROS production and mitochondrial DNA oxidative damage in rat liver mitochondria offering a plausible mechanism for its hepatotoxicity (Gomez et al., 2009) in all the determined essential amino acids, the mean content of leucine was the highest, corresponding the research by Wu et al. (2007).

The highest content of leucine in J. sigillata was 42.04 mg/g (‘Y042’), while in J. regia that was 50.19 mg/g (‘X9’). In addition, the mean values of leucine were 38.49 mg/g (J. sigillata) and 36.88 mg/g (J. regia). As a dietary supplement, leucine has been found to slow the degradation of muscle tissue by increasing the synthesis of muscle proteins in aged rats (Combaret et al., 2005). However, the supplementation of leucine with long-term didn’t increase muscle mass or strength in healthy elderly men (Verhoeven et al., 2009). Until now, the dietary supplement of leucine has not be associated the prime reason for muscular growth or optimal maintenance for the entire population.

Furthermore, phenylalanine is an essential amino acid and required to assure normal anabolism (Pimentel et al., 2014). Phenylalanine as a nutritional supplement is used in the manufacture of food and drink products for its famous functions of analgesic and antidepressant. In this study, the content of phenylalanine in 'Y029' (J. sigillata) was the highest (20.69 mg/g) and the mean value of phenylalanine was 18.76 mg/g. While the highest content of phenylalanine in J. regia was 22.26 mg/g in ‘XJ004’ and the mean level of phenylalanine was 17.44 mg/g. In addition, phenylalanine hydroxylase (PAH) is responsible for converting dietary phenylalanine into tyrosine in the liver. However, when PAH is lacked or changed lowly, people will be dangerous for phenylketonuria (PKU). Therefore, In order to reach safe concentrations of phenylalanine in blood, PKU patients need to follow a Phenylketonuria (Giovannini et al., 2007; Rocha et al., 2012).

Finally, as shown in Tab. 3, the highest content of lysine in J. sigillata was 22.93 mg/g (‘Y021’), while the highest one in J. regia was 29.43 mg/g (‘X9’). Lysine is a precursor compound of arginine and histidine. In view of lysine, the high content of lysine negatively influenced the uptake of arginine in vivo (Wu and Meininger, 2002). Lysine residues were essential for both receptor-dependent proinflammatory and receptor-independent cytolytic activities (Cheung et al., 2014). In comparison, the results for the composition of essential amino acid in J. sigillata and J. regia from China were consistent with several published reports including those for Spanish almonds (Calixto et al., 1981), New Zealand hazelnuts (Savage and McNeil, 1998), Turkish Tombul hazelnut (Alasalvar et al., 2003), Chilean hazelnut (Villarroel et al., 1987), almond, pecan, pine nut, and pistachio (Ruggeri et al., 1998). What's more, it was favorable with the data of amino acid composition for edible nuts from USDA (Tab. 4)(USDA, 2013). It can be seen that the ratio of the contents of essential amino acids in both J. sigillata and J. regia to the data from USDA were more than two-fold, even the highest ratio of isoleucine nearly reached five-fold. Therefore, the kernels of J. sigillata and J. regia were considered to be good sources of essential amino acids. In addition, researchers reported that lysine was the first limiting essential amino acid in total walnut proteins (Ruggeri et al., 1998; Sze-Tao and Sathe, 2000) Compared to the recommended essential amino acid pattern from WHO/FAO for an adult (Joint, 2007), all the determined kernels contain adequate amounts of all the essential amino acids.

Tab. 4. Essential amino acids contents of walnut kernel (mg/g, dry weight) from USDA National Nutrient Database and walnuts (J. regia and J. sigillata)

<table>
<thead>
<tr>
<th>Essential amino acids</th>
<th>USDA</th>
<th>J. sigillata</th>
<th>Ratios (J. sigillata/USDA)</th>
<th>J. regia</th>
<th>Ratios (J. sigillata/USDA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>5.96</td>
<td>16.24</td>
<td>2.72</td>
<td>13.87</td>
<td>2.33</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>6.25</td>
<td>30.53</td>
<td>4.88</td>
<td>30.24</td>
<td>4.84</td>
</tr>
<tr>
<td>Leucine</td>
<td>11.70</td>
<td>38.49</td>
<td>3.29</td>
<td>36.88</td>
<td>3.15</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.36</td>
<td>6.58</td>
<td>2.79</td>
<td>6.28</td>
<td>2.66</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>7.11</td>
<td>18.76</td>
<td>2.64</td>
<td>17.44</td>
<td>2.45</td>
</tr>
<tr>
<td>Valine</td>
<td>7.53</td>
<td>19.53</td>
<td>2.59</td>
<td>15.75</td>
<td>2.09</td>
</tr>
</tbody>
</table>
Conclusions

The kernels of walnuts contained relatively high amounts of Mg, Ca, Zn, Mn, Fe, and Cu, in which Mg and Ca were the major mineral elements. The contents of mineral elements were different between J. regia and J. sigillata. The order of nutritive mineral elements depending on their content (mg/100g) of samples was Mg > Ca > Zn > Mn > Fe > Cu in J. regia, while the order in J. sigillata was Mg > Ca > Mn > Fe > Zn > Cu. In comparison, leucine was the highest content of essential amino acids in all the investigated samples. The order depending on the content (mg/g) of the essential amino acids in J. regia samples was leucine > isoleucine > valine > phenylalanine > lysine > threonine > methionine, while the order in J. sigillata was leucine > isoleucine > lysine > phenylalanine > valine > threonine > methionine. Moreover, ‘Y029’ in Juglans sigillata and ‘XJ004’ in Juglans regia provided the best profiles of mineral elements and essential amino acids in comparison to others. Importantly, the kernel of walnut is a good source of health food and dietary supplement for mineral elements and essential amino acids.

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References


