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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> methionine, 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 various 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*;* Qingzhu *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 Mg 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', 'Körcegöz', '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 ('Esterhazy', '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, *g*-tocopherol 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* ('XJ004', 'XJ005', 'XJ008', 'XJ012', 'XJ017', 'XJ018', 'XJ019', 'XJ020', 'XJ029', 'SX05', 'SX13', 'SS004', 'SAK002', 'SHZ002', 'X1', '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 isothiocyanate (PITC, 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% hydrogen peroxide 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 ultrahigh-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 fassele 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 hydrolysate 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 /PITC (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, Zn, and Cu 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 1.11.

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 ('SS004') 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.

Tab. 1. The contents of mineral elements (mg/100g, dry weight) in kernels from 11 kinds of walnuts (*J. sigillata*) and 17 kinds of walnuts (*J. regia*)

No.	Samples	Mineral Elements					
		Ca	Mg	Fe	Mn	Zn	Cu
	J. sigillata						
1	Y021	266.63 ± 0.18 c	651.33 ± 0.48 f	11.29 ± 0.02 d	13.38 ± 0.03 e	8.85 ± 0.03 b	6.17 ± 0.02 de
2	Y026	268.23 ± 0.32 d	698.87 ± 0.73 i	10.88 ± 0.16 c	12.55 ± 0.04 e	7.66 ± 0.03 a	3.85 ± 0.04 b
3	Y029	282.57 ± 0.45 e	653.13 ± 0.63 fg	10.34 ± 0.06 b	6.84 ± 0.04 b	12.37 ± 0.04 g	5.59 ± 0.05 cd
4	Y041	285.27 ± 0.80 g	625.43 ± 0.38 c	9.44 ± 0.06 a	5.47 ± 0.10 a	10.43 ± 0.17 d	3.65 ± 0.07 b
5	Y042	285.37 ± 0.54 g	653.50 ± 1.02 g	9.71 ± 0.06 a	18.43 ±0.05 f	10.93 ± 0.03 e	5.71 ± 0.06 cd
6	Y075	284.50 ± 0.46 fg	538.40 ± 0.40 a	11.56 ± 0.04 d	24.51 ± 0.07 g	13.63 ± 0.05 h	4.87 ± 0.03 c
7	Y082	283.23 ± 0.44 ef	579.20 ± 0.47 b	13.14 ± 0.09 f	10.29 ± 0.21 d	11.56 ± 0.21 f	5.37 ± 0.07 cd
8	Y096	155.10 ± 0.45 a	771.93 ± 0.46 j	10.67 ± 0.19 c	9.35 ± 0.12 c	10.31 ± 0.07 d	4.96 ± 0.06 c
9	Y098	261.10 ± 0.46 b	640.30 ± 0.32 e	13.72 ± 0.13 g	24.50 ± 0.26 g	10.37 ± 0.10 d	5.72 ± 0.06 cd
10	Y099	318.60 ± 0.45 h	631.20 ±0.43 d	12.55 ± 0.07 e	19.28 ± 0.16 f	11.39 ± 0.10 f	6.91 ± 1.05 e
11	Y101	482.03 ± 0.55 i	680.03 ± 1.19 h	12.33 ± 0.11 e	13.14 ± 0.07 e	9.95 ± 0.06 c	1.46 ± 0.05 a
	J. regia						
1	XJ004	253.90 ± 1.80 a	483.30 ± 0.44 c	10.45 ± 0.08 k	4.46 ± 0.03 c	10.38 ± 0.03 e	3.23 ± 0.04 d
2	XJ005	295.27 ± 0.49 b	614.53 ± 0.30 kl	9.84 ± 0.06 j	5.48 ± 0.04 f	9.64 ± 0.08 d	3.35 ± 0.02 de
3	XJ008	423.37 ± 0.40 k	616.73 ± 0.50 l	8.83 ± 0.03 g	9.01 ± 0.07 l	12.69 ± 0.05 h	5.30 ± 0.06 k
4	XJ012	336.17 ± 0.64 h	557.70 ± 0.44 f	9.58 ± 0.10 i	7 .34 ± 0.07 i	12.55 ± 0.11 h	5.10 ± 0.06 j
5	XJ017	310.57 ± 0.35 e	607.37 ± 0.43 j	8.45 ± 0.07 f	5.12 ± 0.06 e	11.56 ± 0.14 f	4.42 ± 0.04 g
6	XJ018	328.33 ± 0.35 f	567.30 ± 0.26 g	7.86 ± 0.05 e	4.86 ± 0.03 d	13.32 ± 0.05 j	3.43 ± 0.06 e
7	XJ019	341.97 ± 1.35 i	581.27 ± 0.52 h	9.26 ± 0.03 h	8.37 ± 0.08 k	13.13 ± 0.08 ij	4.64 ± 0.03 h
8	XJ020	299.53 ± 0.38 c	642.50 ± 0.96 m	7.26 ± 0.06 d	3.93 ± 0.01 b	12.65 ± 0.05 h	4.86 ± 0.03 i
9	XJ029	295.37 ± 0.64 b	594.13 ± 0.52 i	8.37 ± 0.03 f	5 .88 ± 0.07 g	13.11 ± 0.06 i	5.21 ± 0.05 jk
10	SX05	331.43 ± 0.28 m	525.53 ± 0.58 e	9.97 ± 0.07 j	15.48 ± 0.14 n	12.07 ± 0.07 g	4.15 ± 0.03 f
11	SX13	451.40 ± 0.72 g	739.00 ± 0.56 n	7.05 ± 0.03 c	17.12 ± 0.02 o	12.11 ± 0.05 g	3.42 ± 0.05 e
12	SS004	504.07 ± 0.43 n	566.80 ± 1.82 g	5.85 ± 0.04 b	7.56 ± 0.04 j	12.09 ± 0.01 g	1.37 ± 0.03 a
13	SAK002	326.23 ± 1.88 f	613.07 ± 1.41 k	4.44 ± 0.06 a	2.73 ± 0.06 a	7.93 ± 0.06 c	3.48 ± 0.05 e
14	SHZ002	302.73 ± 0.81 d	479.97 ± 1.24 b	7.34 ± 0.09 d	10.53 ± 0.05 m	7.12 ± 0.05 b	2.11 ± 0.05 b
15	X1	448.40 ± 1.01 l	401.47 ± 0.38 a	5.74 ± 0.03 b	15.55 ± 0.05 n	6.84 ± 0.04 a	2.17 ± 0.03 b
16	X9	405.00 ± 0.43 j	506.20 ± 0.43 d	9.84 ± 0.04 j	9.13 ± 0.04 l	12.69 ± 0.06 h	4.11 ± 0.05 f
17	SS002	333.27 ± 0.64 g	749.27 ± 0.75 o	8.72 ± 0.05 g	6.26 ±0.03h	9.61 ± 0.06 d	2.95 ±0.03c

The same letter indicated that the values were not significantly different at P < 0.05, according to Duncan's multiple range test.

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 13.32 mg/100g ('XJ018') in *J. regia*, 7.66 ('Y026') and 13.60 mg/100g ('Y075') in *J. sigillata* (Tab. 1). The mean values of Zn were 11.15 (*J. regia*) and 10.68 mg/100g (*J. sigillata*). As the second most abundant transition metal ion after iron in the human body, Zn is believed to be an essential factor in many biological processes such as brain function and pathology, gene transcription, immune function, and mammalian reproduction (Vallee and Falchuk, 1993; Berg and Shi, 1996; Xu *et al.*, 2010).

The contents of Mn varied between 2.73 mg/100g in 'SAK002' and 17.12 mg/100g in 'SX13' from *J. regia*, 5.47 mg/100g in 'Y075' and 24.51 mg/100g in 'Y075' from *J. sigillata*. In addition, the mean contents of Mn were 8.17 mg/100g in *J. regia* and 14.34 mg/100g in *J. sigillata*. The ratio of the mean contents of Mn in *J. sigillata* to *J. regia* was 1.76. In human body, Mn is an important glandular regulator and involved in the metabolism of glucydes, lypides, and protides. At the same time, Mn plays an important physiological role in the nervous system and cardiac function (Ding *et al., 2014*).

It is well known that Fe is a key kind of element for health and normal performance of organism. In this paper, the contents of Fe were between 4.44 mg/100g in 'SAK002' and 10.45 mg/100g in 'XJ004' from *J. regia*, 9.44 mg/100g in 'Y041' and 13.72 mg/100g in 'Y098' from *J. sigillata*. The mean values of Fe 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 ('Y099') 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 leucine> isoleucine> valine> phenylalanine> lysine> threonine> methionine, while the order in *J. sigillata* was leucine> isoleucine> lysine> phenylalanine> valine> threonine> methionine.

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*)

Minerals	USDA				Walnuts (*J. regia* and *J. sigillata*)			
	Units	Value per 100 grams	No. of Data Points	Std. Error	Units	Value per 100 grams	No. of Data Points	Std. Error
Ca	mg	98	7	3.009	mg	327.13	28	1.572
Fe	mg	2.91	7	0.086	mg	9.45	28	0.424
Mg	mg	158	7	1.204	mg	606.05	28	1.885
Zn	mg	3.09	7	0.083	mg	10.96	28	0.368
Cu	mg	1.586	7	0.026	mg	4.20	28	0.265
Mn	mg	3.414	7	0.122	mg	10.59	28	1.138

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. regia*)

NO.	Samples	Essential amino acid compositions						
		Threonine	Valane	Methionine	Isoleucine	Leucine	Phenylalanine	Lysine
	J. sigillata							
1	Y021	17.65±0.0021 i	4.45±0.0012 a	7.73±0.0012 i	29.08±0.0018 d	38.17±0.0019 e	20.24±0.0018 j	22.93±0.0015 g
2	Y026	13.62±0.0019 a	16.76±0.0033 b	5.47±0.0018 a	25.17±0.0017 a	32.54±0.0023 a	14.55±0.0023 a	18.34±0.0021 cd
3	Y029	17.68±0.0015 i	23.47±0.0021 k	6.16±0.0023 c	33.18±0.0032 k	41.64±0.0018 i	20.69±0.0015 k	19.72±0.0012 ef
4	Y041	16.33±0.0018 f	20.14±0.0018 d	6.73±0.0012 f	31.62±0.0012 g	38.90±0.0094 f	19.18±0.0023 f	18.77±0.0026 de
5	Y042	16.78±0.0018 g	22.39±0.0012 i	6.66±0.0018 e	32.73±0.0023 i	42.04±0.0018 j	20.07±0.0038 i	22.12±0.0015 g
6	Y075	18.32±0.0026 j	23.41±0.0023 j	7.05±0.0019 h	32.85±0.0026 j	40.69±0.0021 h	19.94±0.0020 h	19.10±0.0018 def
7	Y082	14.36±0.0015 b	20.79±0.0012 e	6.53±0.0023 d	30.92±0.0023 e	38.03±0.0019 d	18.16±0.0029 e	15.45±0.0027 b
8	Y096	15.34±0.0018 d	21.25±0.0023 f	5.93±0.0019 b	28.34±0.0018 b	35.35±0.0023 b	17.93±0.0012 c	13.43±0.0993 a
9	Y098	17.42±0.0009 h	21.68±0.0015 h	6.65±0.0023 e	31.96±0.0029 h	40.35±0.0015 g	19.74±0.0020 g	18.77±0.0023 de
10	Y099	15.97±0.0020 e	21.34±0.0021 g	6.82±0.0026 g	31.02±0.0026 f	38.98±0.0015 f	18.08±0.0017 d	19.79±0.0015 f
11	Y101	15.14±0.0018 c	19.14±0.0015 c	6.62±0.0020 e	28.92±0.0026 c	36.67±0.0020 c	17.73±0.0015 b	17.80±0.0028 c
	J. regia							
1	XJ004	17.72± 0.0032 o	23.93± 0.0035 p	7.47±0.0021 l	33.53±0.0012 o	42.93± 0.0026 o	22.26± 0.0024 p	22.33± 0.0021 l
2	XJ005	12.98±0.0020 d	18.11±0.0026 m	5.33±0.0019 a	26.22±0.0023 c	32.98±0.0029 d	15.74±0.0017 c	20.49±0.0029 h
3	XJ008	13.19±0.0015 e	16.29±0.0018 g	6.49±0.0029 g	29.29±0.0012 k	35.73±0.0019 g	17.34±0.0015 i	19.69±0.0015 e
4	XJ012	11.12±0.0045 a	13.54±0.0018 c	7.12±0.0026 k	22.61±0.0012 a	31.02±0.0029 a	14.94±0.0027 a	16.91±0.0026 b
5	XJ017	13.75±0.0020 g	17.57±0.0012 k	6.23±0.0023 e	29.76±0.0026 l	36.01±0.0023 i	16.98±0.0015 f	19.97±0.0032 f
6	XJ018	13.88±0.0017 h	17.25±0.0010 i	6.81±0.0015 j	28.19±0.0017 g	36.28±0.0032 j	17.47±0.0020 j	20.10±0.0012 g
7	XJ019	13.27±0.0026 f	15.73±0.0012 e	5.63±0.0015 b	27.98±0.0009 f	34.75±0.0009 e	17.54±0.0015 k	19.48±0.0015 d
8	XJ020	11.97±0.0009 c	15.98±0.0025 f	5.34±0.0018 a	24.54±0.0018 b	32.23±0.0015 b	15.88±0.0017 d	20.71±0.0029 i
9	XJ029	12.99±0.0026 d	16.95±0.0012 h	6.74±0.0018 i	28.51±0.0020 i	35.74±0.0024 g	17.04±0.0015 g	21.32±0.0024 j
10	SX05	14.10±0.0015 i	17.35±0.0012 j	6.24±0.0015 e	29.93±0.0022 m	37.14±0.0018 k	18.54±0.0025 m	18.88±0.0024 c
11	SX13	14.44±0.0025 j	17.56±0.0009 k	6.04±0.0015 d	29.23±0.0027 j	37.74±0.0012 l	18.62±0.0023 n	20.03±0.0015 fg
12	SS004	13.13±0.0019 e	14.62±0.0020 d	5.73±0.0019 c	26.31±0.0015 d	32.65±0.0022 c	16.04±0.0015 e	16.78±0.0021 a
13	SAK002	15.68±0.0015 m	18.34±0.0015 o	6.60±0.0033 h	27.19±0.0012 e	35.81±0.0023 h	17.25±0.0023 h	22.22±0.0026 k
14	SHZ002	15.76±0.0009 n	18.26±0.0018 n	6.49±0.0015 g	28.29±0.0015 h	35.61±0.0026 f	18.22±0.0033 l	22.23±0.0021 k
15	X1	11.30±0.0021 b	2.74±0.0018 a	5.58±0.0018 b	44.46±0.0024 p	41.96±0.0020 n	15.48±0.0013 b	27.47± 0.0026 m
16	X9	15.50±0.0018 l	5.54±0.0015 b	6.63±0.0032 h	47.53±0.0012 q	50.19±0.0441 p	18.85±0.0023 o	29.43±0.0023 n
17	SS002	15.09±0.0023 k	17.92±0.0035 l	6.34±0.0018 f	30.43±0.0009 n	38.26±0.0026m	18.25±0.0022 l	21.30±0.0018 j

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 aline 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 value 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 supplemental of leucine has not been 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 Phe-restricted diet (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*)

Essential amino acids	USDA	*J. sigillata*	*J. regia*	Ratios	
				J. sigillata/USDA	*J. sigillata*/USDA
Threonine	5.96	16.24	13.87	2.72	2.33
Isoleucine	6.25	30.53	30.24	4.88	4.84
Leucine	11.70	38.49	36.88	3.29	3.15
Lysine	4.24	18.75	21.14	4.42	4.99
Methionine	2.36	6.58	6.28	27.9	2.66
Phenylalanine	7.11	18.76	17.44	2.64	2.45
Valine	7.53	19.53	15.75	2.59	2.09

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 good source of health food and dietary supplement for mineral elements and essential amino acids.

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References

Alasalvar C, Shahidi F, Liyanapathirana CM, Ohshima T (2003). Turkish tombul hazelnut (*Corylus avellana* L.). 1. Compositional characteristics. J Agric Food Chem 51(13):3790-3796.

Albuquerque TG, Costa HS, Sanches-Silva A, Santos M, Trichopoulou A, D'Antuono F, Alexieva I, Boyko N, Costea C, Fedosova K (2013). Traditional foods from the Black Sea region as a potential source of minerals. J Sci Food Agric 93(14):3535-3544.

Bryant RJ, Cadogan J, Weaver CM (1999). The new dietary reference intakes for calcium: implications for osteoporosis. J Am Coll Nutr 18(Sup5):406S-412S.

Caglarirmak N (2003). Biochemical and physical properties of some walnut genotypes (*Juglans regia* L.). Food/Nahrung, 47(1):28-32.

Calixto FS, Bauza M, Martinez de Toda F, Argamenteria A (1981). Amino acids, sugars, and inorganic elements in the swe*et al*mond (*Prunus amygdalus*). J Agric Food Chem 29(3):509-511.

Cheung GY, Kretschmer D, Queck SY, Joo H-S, Wang R, Duong AC, Nguyen TH, Bach T-HL, Porter AR, DeLeo FR (2014). Insight into structure-function relationship in phenol-soluble modulins using an alanine screen of the phenol-soluble modulin (PSM) α3 peptide. FASEB J 28(1):153-161.

Combaret L, Dardevet D, Rieu I, Pouch M-N, Béchet D, Taillandier D, Grizard J, Attaix D (2005). A leucine-supplemented diet restores the defective postprandial inhibition of proteasome-dependent proteolysis in aged rat skeletal muscle. J Physiol 569(2):489-499.

Ding Y, Luo Y, Fu J (2014). Effects of Mn (II) on peroxynitrite nitrifying fibrinogen. Bio-med Mater Eng 24(1):901-907.

Fraga CG (2005). Relevance, essentiality and toxicity of trace elements in human health. Mol Aspects Med 26(4):235-244.

Fürst P, Stehle P (2004). What are the essential elements needed for the determination of amino acid requirements in humans? J Nutr 134(6):1558S-1565S.

Giovannini M, Verduci E, Salvatici E, Fiori L, Riva E (2007). Phenylketonuria: dietary and therapeutic challenges. J Inherit Metab Dis 30(2):145-152.

Gomez J, Caro P, Sanchez I, Naudi A, Jove M, Portero-Otin M, Lopez-Torres M, Pamplona R, Barja G (2009). Effect of methionine dietary supplementation on mitochondrial oxygen radical generation and oxidative DNA damage in rat liver and heart. J Bioenerg Biomembr 41(3):309-321.

Hao Y, Wang K, Wang S, Zhuang Y (2002). Analysis of the protein and fat among pricocious varieties of walnut. Food Science 23(10):123-125.

Imura K, Okada A (1998). Amino acid metabolism in pediatric patients. Nutrition 14(1):143-148.

Joint W (2007). Protein and amino acid requirements in human nutrition. World Health Organization Technical Report Series 935:1-265.

Lavedrine F, Ravel A, Villet A, Ducros V, Alary J (2000). Mineral composition of two walnut cultivars originating in France and California. Food Chem 68(3):347-351.

Martínez ML, Labuckas DO (2010). Lamarque AL and Maestri DM, Walnut (*Juglans regia* L.): genetic resources, chemistry, by-products. J Sci Food Agric 90(12):1959-1967.

Nash SD, Westpfal M (2005). Cardiovascular benefits of nuts. Am J Cardiol 95(8):963-965.

Özcan MM, İman C, Arslan D (2010). Physico-chemical properties, fatty acid and mineral content of some walnuts (*Juglans regia* L.) types. Agricultural Sciences 1(2):62-67.

Ozrenk K, Javidipour I, Yarilgac T, Balta F, Gündogdu M (2012). Fatty acids, tocopherols, selenium and total carotene of pistachios (*P. vera* L.) from Diyarbakir (Southeastern Turkey) and walnuts (*J. regia* L.) from Erzincan (Eastern Turkey). Food Sci Technol Int 18(1):55-62.

Patel G (2005). Essential fats in walnuts are good for the heart and diabetes. J Am Diet Assoc 105(7):1096-1097.

Pimentel FB, Alves RC, Costa AS, Fernandes TJ, Torres D, Almeida MF, Oliveira M (2014). Nutritional composition of low protein and phenylalanine-restricted dishes prepared for phenylketonuric patients. LWT-Food Sci Technol 57(1):283-289.

Qin Z, Guo X, Lin Y, Chen J, Liao X, Hu X, Wu J (2012). Effects of high hydrostatic pressure on physicochemical and functional properties of walnut (*Juglans regia* L.) protein isolate. J Sci Food Agric 93(5):1105-1111.

Zhang Q, Ding X, Cheng Z, Kan J, Zhao G (2003). The development of research on walnut protein. Journal of Cereals and Oils 5:21-23.

Reeds PJ (2000). Dispensable and indispensable amino acids for humans. J Nutr 130(7):1835S-1840S.

Rocha JC, Spronsen FJV, Almeida MF, Soares G, Quelhas D, Ramos E, Guimarães JT, Borges N (2012). Dietary treatment in phenylketonuria does not lead to increased risk of obesity or metabolic syndrome. Mol Genet Metab 107(4):659-663.

Ruggeri S, Cappelloni M, Gambelli L, Nicoli S, Carnovale E (1998). Chemical composition and nutritive value of nuts grown in Italy. Ital J Food Sci 10(3):243-252.

Sabate J, Fraser GE, Burke K, Knutsen SF, Bennett H, Lindsted KD (1993). Effects of walnuts on serum lipid levels and blood pressure in normal men. New Engl J Med 328(9):603-607.

Savage G (2001). Chemical composition of walnuts (*Juglans regia* L.) grown in New Zealand. Plant Food Hum Nutr 56(1):75-82.

Savage G, McNeil D (1998). Chemical composition of hazelnuts (*Corylus avellana* L.) grown in New Zealand. Int J Food Sci Nutr 49(3):199-203.

Spies JR, Chambers DC (1948). Chemical determination of tryptophan. Anal Chem 20(1):30-39.

Sze-Tao KWC, Sathe SK (2000). Walnuts (*Juglans regia* L): proximate composition, protein solubility, protein amino acid composition and protein in vitro digestibility. J Sci Food Agric 80(9):1393-1401.

Tapiero H, Tew KD (2003). Trace elements in human physiology and pathology: zinc and metallothioneins. Biomed Pharmacothe 57(9):399-411.

Tapiero H, Townsend D, Tew K (2003). Trace elements in human physiology and pathology. Copper. Biomed Pharmacother 57(9):386-398.

Touyz RM (2004). Magnesium in clinical medicine. http://www.ncbi.nlm.nih.gov/pubmed/14977544.

U.S. Department of Agriculture Research Service (2013). [Online]. USDA National Nutrient Database for Standard Reference, Release 26. Nutrient Data Laboratory Home. Available: ttp://www.ars.usda.gov/Services/docs.htm?docid=8964.

Vallee BL, Falchuk KH (1993). The biochemical basis of zinc physiology. Physiol Rev 73(1):79-118.

Verhoeven S, Vanschoonbeek K, Verdijk LB, Koopman R, Wodzig WK, Dendale P, van Loon LJ (2009). Long-term leucine supplementation does not increase muscle mass or strength in healthy elderly men. Am J Clin Nutr 89(5):1468-1475.

Villarroel M, Biolley E, Schneeberger R, Ballester D, Ramirez S (1987). Amino acid composition of Chilean hazel nuts. Food Chem 25(2):155-158.

Warmund MR, Elmore J, Drake M, Yates MD (2009). Descriptive analysis of kernels of selected black and Persian walnut cultivars. J Sci Food Agric 89(1):117-121.

Wood J, Decker H, Hartmann H, Chavan B, Rokos H, Spencer J, Hasse S, Thornton M, Shalbaf M, Paus R (2009). Senile hair graying: H_2O_2-mediated oxidative stress affects human hair color by blunting methionine sulfoxide repair. FASEB J 23(7):2065-2075.

Wu G, Meininger CJ (2002). Regulation of nitric oxide synthesis by dietary factors. Annu Rev Nutr 22(1):61-86.

Wu K, Xiao Q, Tang L, Pang Y, Wei L, Zhang L (2007). Differences analysis on the contents of crude fat and amino acids in kernel of walnut. Nonwood Forest Research 25(2):15-18.

Xie X, Smart TG (1991). A physiological role for endogenous zinc in rat hippocampal synaptic neurotransmission. Nature 349:521-524.

Xu Z, Yoon J, Spring DR (2010). Fluorescent chemosensors for Zn^{2+}. Chem Soc Rev 39(6):1996-2006.

Young VR (1994). Adult amino acid requirements: the case for a major revision in current recommendations. J Nutr 124(Sup8):1517S.