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The Nutritional Qualities of Walnuts and Their Planted Soils from China—Level and Relationship

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Abstract: China is the world's largest walnut producer. There is still a scarcity of comprehensive information on the nutritional quality of Chinese walnuts and the influence of soil on walnut quality. In this study, 273 samples and the corresponding soils from the main English walnut (*Juglans regia* L.)-producing areas in China were collected. The results revealed that walnut oil contained a high percentage of linoleic acid and γ-tocopherol, with an average content of 62.36% and 77.70%, respectively. Walnut flour is rich in nutritional elements, with the highest content of macro-element K at 3961.29 mg kg^{-1}. In addition, 12 micro-elements with concentrations ranging from 0.01 to 42.34 mg/kg were discovered in walnut flour. The total amino acid profile of walnut flour contains 27.23% of the seven essential amino acids, with the highest amount allocated to leucine at 2.07 g 100 g^{-1}. The Pearson correlation analysis revealed that almost all amino acids showed a significant positive correlation ($p < 0.01$, $r > 0.70$). The soil's content in terms of three trace elements (Fe, Cu, and Zn) has the greatest influence on the nutritional qualities of walnuts. Furthermore, the pH of the soil played a role in determining the qualities of the walnut. These findings provide fundamental information for the consumer selection and trade of walnuts, as well as guidance for the development of high-quality walnuts in China.

Keywords: walnut; nutritional quality; fatty acid; element; amino acid; soil effect

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1. Introduction

Walnuts are widely consumed as snacks or as ingredients in a range of food products around the world. Studies have shown that eating walnuts can help prevent cancer, diabetes, and some cardiovascular and cerebrovascular disease [1,2]. Walnuts represent a calorie-rich, nutrient-dense food, mainly due to their high lipid content. Many studies have demonstrated the advantage of walnuts over other tree nuts in terms of unsaturated fatty acid content [3,4]. Moreover, according to data from the US Department of Agriculture's National Nutrition Service, walnuts contain 6 and 59 times more polyunsaturated fatty acids than cashews, chestnuts, and hazelnuts [5]. The majority of walnut composition research has concentrated on the lipid fraction, specifically fatty acids (FAs). Many previous studies concentrated on the content of five major FAs in walnut varieties collected from the same or a few different orchards [6–13]. They were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3). These FAs have been shown to benefit human health by lowering the risk of cardiovascular disease and improving cognitive function [14]. Aside from the variety, climatic changes, and differing cultivation altitudes associated with walnut, geographic origin has been linked to fluctuations in FAs content [15]. Many scholars have conducted research on walnut FAs on a regional or national scale, and the results of these studies are important for consumer choice and trade, as well as walnut quality regulation [16].

China is the world's largest walnut producer, accounting for more than half of total walnut output. Some researchers (including ourselves) have published studies on the FAs in some of China's most popular varieties of walnut [11,13,17]. However, there is a lack of information on the quality differences between Chinese and other nations' walnuts, and more research on the relationship between the quality (rather than just fatty acid) components of Chinese walnuts is needed. Furthermore, walnut flour (WF), a byproduct of walnut oil extraction, contains a high concentration of bioactive compounds, such as micronutrients and amino acids. The consumption of these components has been shown to reduce the risk of diabetes and other disorders [18], particularly in areas where nuts and vegetables are scarce. There are, however, few reports on the nutritional compositions of WF [19].

Moreover, it has been demonstrated that soil has a significant impact on the nutritional quality of food. There is a lot of study being carried out on the relationship between the physical and chemical qualities of the soil in growing areas and the nutritional quality of fruits. Soil properties accounted for up to 91% of the difference in nutritional quality of kiwifruit [20]. According to one study, selecting appropriate micronutrient fertilizer combinations could help improve nutrient deficiency in Camellia oleifera [21]. Growing passion fruit in soil with a lower pH (pH = 4.5) has been shown to improve nutritional quality [22]. Despite previous research on the nutritional quality of walnuts in various locations, research on the relationship between soil qualities and walnut nutritional quality remains limited. As a result, more study is required to close this knowledge gap. The primary goal of this study is to investigate the nutritional quality of walnuts in China's main growing regions (English walnut production accounted for more than 64% of total production), as well as to evaluate the quality characteristics of Chinese walnuts. Our study will look into the impact of soil qualities on the nutritional quality of walnuts, with the goal of better understanding the relationship between soil quality and food nutritional value.

2. Materials and Methods

2.1. Chemicals

HPLC grade reagents (isooctane, hexane, isopropanol, and methanol) and standard reagents (gallic acid, α-tocopherol, δ-tocopherol, γ-tocopherol) were purchased from Merck (Darmstadt, Germany). Sodium nitrite, sodium carbonate, hydrogen nitrate, aluminum nitrate, and hydrochloric acid were purchased from Shanghai Guoyao Chemical Reagent (Shanghai, China). The National Analysis Center of Iron and Steel (Beijing, China) provided multi-element standard solutions containing 100 mg L^{-1} of each element.

2.2. Sample Collection

During two consecutive nut harvest seasons, 273 walnut (*Juglans regia* L.) samples were collected from local orchards in eight Chinese provinces: Shaanxi (37), Shanxi (26), Xinjiang (55), Henan (28), Hebei (48), Liaoning (20), Gansu (39), and Shandong (20). The production of English walnuts in these eight provinces accounted for more than 64% of the total production of China. The detailed information for the collection locations can be found in the Supplementary File (Figure S1 and Table S1). In order to obtain a representative sample, 10–15 nuts were collected from different randomly selected nut trees, and the process was repeated 10 times at the same sampling site. Walnut samples were mixed well to obtain a representative sample (>1 kg) from each sampling site. They were then quickly transported back to the laboratory for drying. The nuts were manually cracked and shelled to obtain the walnut kernels. The walnut kernels were ground to a powder using a grinder and stored at −20 °C.

The soil samples corresponding to the walnut samples were collected from the soil around the walnut trees. The corresponding soil samples (0–20 cm) were collected using a soil auger. Multiple soil samples (>1 kg) were collected from randomly selected locations at each site and mixed to obtain a representative sample.

2.3. Nutritional Components Analysis: Walnut

2.3.1. Fatty Acid

Walnut oil (WO) was extracted from crushed walnut kernels through solvent extraction.

Fatty acid methyl esters (FAME) from the WO were obtained by alkaline treatment with 2.0 mol L^{-1} KOH in methanol. The analysis was performed in a 7890A gas chromatographic method (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (GC-FID) and a capillary column (HP-INNOW AX, 30 m × 0.32 mm × 0.25 μm). Detailed operating conditions are described in previous work [18]. C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, and C20:1 represent palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, and eicosenoic acid, respectively.

2.3.2. Tocopherol

WO (0.3 g) was dissolved in 5 mL of *n*-hexane and vortexed for 30 s. The mixture was filtered through a 0.45 μm nylon filter and analyzed for tocopherol using high-performance liquid chromatography (HPLC) (Agilent Technologies, Palo Alto, CA, USA) and a Prodigy ODS-2 column (2 × 150 mm, 5 μm). The details of the analysis procedure were described in our previous work [23].

2.3.3. Mineral Elements

Walnut flour (WF) is obtained by removing the oil from walnut kernels. Sixteen mineral elements in WF were analyzed via ICP-OES (iCAP 7000) (K, Ca, Na, Mg, Fe, Cu, Zn, and P) and ICP-MS (NexIon 300D, Perkin Elmer, Shelton, CT, USA) (B, Ba, Co, Mo, Ni, Se, Ti, and V), respectively.

2.3.4. Amino Acids

The amino acid content of WF was determined by automated amino acid analysis using an L-8900 (L-8900, Hitachi High Technologies Corporation. Tokyo, Japan). Details of the operation can be found in the Supplementary File.

2.4. Soil Samples Analysis

Fifteen mineral elements in soils were analyzed via ICP-OES (iCAP 7000) (K, Na, Mg, Fe, Cu, Zn, and P) and ICP-MS (NexIon 300D, Perkin Elmer, Shelton, CT, USA) (Mn, Ba, Co, Mo, Ni, Cr, Pb, and Cd), respectively. The detailed process of soil property (pH and OM) analysis can be seen in our previous work [24].

2.5. Statistical Analysis

SPSS (version 19.0, IBM, Armonk, NY, USA) was used to process the data for the chemical components in analyzed samples. A Pearson correlation analysis was performed between soil properties, mineral elements, and walnut chemical components using a statistical package program (Pearson correlation analysis was performed by using Corrplot tools in Hiplot Pro at https://hiplot.com.cn (accessed on 1 February 2023), a comprehensive web service for biomedical data analysis and visualization). The correlative network was visualized with Gephi (Version 0.9.2) using the classic Fruchterman Reingold algorithm.

3. Results and Discussion

3.1. Walnut Oil

Walnut oil is a nutrient-rich vegetable oil high in polyunsaturated and monounsaturated FAs. Table 1 shows the results of the fatty acids in walnut oil from this study and other relevant literature (the data were all collected from a specific number of samples). The primary polyunsaturated fatty acid found in Chinese walnut oil is C18:2, accounting for 62.36% of the total. C18:1, a monounsaturated fatty acid associated with health, is the second most abundant fatty acid in Chinese walnut oil, accounting for 18.87% of the total. This C18:2 level in this study is much greater than many of the results reported in the literature, where most of the C18:2 values were less than 60% (details can be found in Table 1). However, no significant differences in C18:1 content distribution were found among the three oil extraction modes. In addition, the C18:3 levels in plant-based foods are rarely high. Walnuts are distinguished from other monounsaturated fatty acid-rich crops by their high C18:3 content (10.02%). Almonds, olives, and sunflower seeds have a significantly lower C18:3 content (all less than 1%) [25].

Previous research has found that the FAs concentration of walnut oil varies depending on the extraction mode [16]. Generally, FAs contents, particularly C18:2 and C18:3 content, were found to be greater in cold-pressed oils than in Soxhlet-extracted oils [26] and in cold solvent extraction [27]. However, we discovered two interesting phenomena by comparing prior research regarding different walnut oil extraction modes, as shown in Table 1. The C18:2 concentration of cold-pressed oil is not always higher than that of oil extracted using the other two methods (solvent and Soxhlet). In terms of C18:2 performance, Chinese walnut oil has a slight advantage based on the mean, maximum, and lowest values of the data.

Besides FAs, certain minor chemicals, such as tocopherol, provide many of the nutritional and physiological benefits of nut oil components. Three tocopherol forms (α-, γ-, and δ-) were detected in this study, with average contents of 2.19, 30.98, and 6.70 mg 100 g^{-1}, respectively. The results clearly demonstrate that γ-tocopherol is the most abundant tocopherol in walnut oil, accounting for more than 77% of the total. Similar findings have been observed in walnut studies from the US [12] and Serbia [10], whereas α-tocopherol is predominant in hazelnut, pistachio, almond, and groundnut oils [28]. Furthermore, we discovered that walnuts are well adapted for usage as a natural source of γ-tocopherols in food or supplements when compared to other nuts, such as almonds (0.50 to 10.40 mg 100 g^{-1}), cashews (4.80 to 5.30 mg 100 g^{-1}), and hazelnuts (4.30 to 9.40 mg 100 g^{-1}) [5].

3.2. Elements in Walnut Flour

The mineral concentration in the proximate profile of WF is critical to its nutritional value, especially when WF is employed as a baking material. Table 2 shows the mineral composition of WF, as well as the mineral composition of a wheat flour and a rice flour, as well as the recommended nutrient intake (RNI) for males and females (19–65 years old), and the percentage of RNI provided by 100 g of WF. The four macro-elements contained 3961.29, 3340.02, 1634.23, and 1305.83 mg kg^{-1} of potassium (K), phosphorous (P), magnesium (Mg), and calcium (Ca), respectively. The 12 micro-elements ranged from 0.01 to 42.34 mg kg^{-1}, with manganese (Mn) having the highest concentrations and vanadium (V) having the lowest. Table 2 shows the mean values for the mineral composition of walnut flour in China, and the mineral composition of walnut flour from the eight provinces can be found in the Supplementary File (Table S2).

Table 1. Comparison of fatty acid content in various walnut-producing regions.

Country/Province	N	C16:0 (%)			C18:0 (%)			C18:1 (%)			C18:2 (%)			C18:3 (%)			Oil Ext. Mode
	Mean	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	
China [A] (Present study)	273	5.73 ± 0.65	4.51	7.28	2.88 ± 0.52	1.90	4.43	18.87 ± 3.84	11.90	28.89	62.36 ± 4.14	52.51	68.83	10.02 ± 1.79	6.37	13.55	Solvent
Shaanxi [B] (Present study)	37	5.67 ± 0.41	5.21	6.79	3.17 ± 0.29	2.26	3.36	16.79 ± 3.18	13.59	27.72	64.83 ± 3.09	52.78	67.23	12.84 ± 1.05	8.33	13.29	Solvent
Shanxi [B] (Present study)	26	5.78 ± 0.51	4.67	6.88	2.91 ± 0.44	2.16	3.81	21.92 ± 4.04	14.60	28.89	59.63 ± 3.20	54.22	65.34	9.43 ± 1.25	6.68	12.31	Solvent
Xinjiang [B] (Present study)	55	6.17 ± 0.50	5.24	7.28	2.64 ± 0.04	2.05	3.49	19.57 ± 3.82	12.69	23.45	60.93 ± 3.26	52.51	68.54	10.46 ± 2.38	6.51	13.55	Solvent
Henan [B] (Present study)	28	5.36 ± 0.46	4.64	6.22	2.93 ± 0.33	2.11	3.78	17.15 ± 3.01	12.32	25.35	64.82 ± 2.41	59.16	68.42	9.63 ± 0.83	7.71	11.54	Solvent
Hebei [B] (Present study)	48	5.59 ± 0.70	4.51	7.06	2.91 ± 0.67	1.92	4.06	18.72 ± 3.46	14.13	27.72	61.38 ± 6.12	53.84	68.83	8.90 ± 1.78	6.73	12.78	Solvent
Liaoning [B] (Present study)	20	5.69 ± 0.32	4.94	6.34	3.43 ± 0.41	2.86	4.43	21.38 ± 5.29	11.90	24.49	59.77 ± 3.78	53.73	66.95	9.40 ± 1.59	6.42	12.31	Solvent
Gansu [B] (Present study)	39	5.61 ± 0.91	4.58	7.13	2.29 ± 0.27	1.90	3.01	19.19 ± 2.50	14.62	26.06	62.90 ± 2.66	57.51	68.01	9.88 ± 1.41	6.44	12.90	Solvent
Shandong [B] (Present study)	20	5.68 ± 0.41	5.15	6.20	3.42 ± 0.28	3.06	3.99	16.46 ± 2.94	12.60	21.13	65.71 ± 1.78	62.31	68.29	8.48 ± 1.25	6.37	10.37	Solvent
Serbia [10]	5	7.06	6.70	7.40	1.78	1.60	2.20	19.90	16.20	22.90	59.66	57.20	63.30	11.12	9.90	13.60	Solvent
Turkey [8]	10	3.70 ± 0.25	1.80	4.74	1.53 ± 0.19	1.17	2.22	24.74 ± 2.71	20.70	28.33	55.40 ± 2.98	50.24	60.60	12.66 ± 1.49	10.93	15.04	Soxhlet
Spain [9]	4	6.83 ± 0.30	6.40	7.10	2.45 ± 0.22	2.20	2.60	15.79 ± 1.17	13.07	17.66	60.00 ± 3.35	59.00	60.60	14.60 ± 0.95	13.60	17.40	Soxhlet
China [13]	37	6.10 ± 0.66	4.98	8.73	2.67 ± 0.27	2.11	3.74	22.12 ± 1.96	11.78	37.39	58.55 ± 2.81	44.22	66.48	9.58 ± 0.95	6.50	12.33	Soxhlet
US [12]	10	6.39 ± 0.71	5.82	6.98	3.65 ± 0.36	3.23	3.97	17.84 ± 1.45	12.95	27.57	60.33 ± 3.07	53.42	64.56	11.26 ± 1.13	7.83	13.16	Soxhlet
China [11]	12	5.97 ± 0.43	4.61	7.91	2.23 ± 0.42	1.42	3.73	19.30 ± 2.23	13.84	35.08	64.13 ± 3.45	52.28	68.87	8.72 ± 0.92	5.04	10.58	Press
Argentina [6]	6	7.12 ± 0.63	5.78	9.70	3.16 ± 0.47	2.47	5.79	16.82 ± 1.73	14.51	20.81	58.61 ± 2.98	52.17	60.93	14.24 ± 1.23	11.19	16.63	Press
Spain [14]	20	7.44	6.87	8.11	3.09	2.38	4.04	22.82	14.56	32.32	55.42	47.49	62.13	10.52	8.21	13.09	Press

Note: N: number of samples; Oil Ext Mode: oil extraction mode; Mean: mean value; Max: maximum value; Min: minimum value; SD: standard deviation values; Solvent: solvent extraction method; Soxhlet: Soxhlet extraction method; press: cold-pressed method; Shannxi: Shannxi Province, China; Shanxi: Shanxi Province, China; Xinjiang: Xinjiang Province, China; Henan: Henan Province, China; Hebei: Hebei Province, China; Liaoning: Liaoning Province, China; Gansu: Gansu Province, China; Shandong: Shandong Province, China; [A]: the total sample obtained in China; [B]: the sample for each province.

Table 2. Comparison of mineral compositions from analyzed walnut flours and other flours.

	Element	Walnut Flour (mg kg^{-1})	Wheat Flour [29] (mg kg^{-1})	Rice Flour [30] (mg kg^{-1})	Recommended Nutrient Intakes (RNI) (mg)	Percentage of RNI Provided by 100 g of WF (%)
Macro-elements	Ca	1305.83 ± 304.57	660	58.10	1000	13.06
	Mg	1634.23 ± 162.79	210	387.60	260	62.85
	K	3961.29 ± 638.69	900	1116.60	4700	8.43
	P	3340.02 ± 473.31	830	1094.80	700	47.71
Micro-elements	Na	11.50 ± 9.02	20	17.30	2400	0.05
	Mn	42.34 ± 26.20	6.00	8.40	2.30	184.09
	Fe	33.03 ± 10.28	/	6.90	14	23.59
	Cu	15.22 ± 3.42	3.30	2.50	0.90	169.33
	Zn	22.20 ± 5.82	8.00	20.40	7.00	31.71
	B	11.57 ± 3.24	/	/	/	/
	Mo	0.31 ± 0.21	/	/	/	/
	Ni	1.56 ± 0.82	/	/	/	/
	Cr	0.36 ± 0.19	/	/	/	/
	Co	0.09 ± 0.04	/	/	/	/
	Se	0.04 ± 0.03	/	/	0.04	11.76
	V	0.01 ± 0.01	/	/	/	/

Note: /: no information available.

When the K content in walnuts from different regions [31–34] is compared, we can see that, while the content of K varies between regions, K is the most abundant element among all the elements measured. In addition, the majority of the analyzed elements in WF are higher than those in the wheat and rice flours. The amount of Ca in WF, in particular, is more than 22 times that of rice flour. This means that WF is a good supply of elemental Ca and P, with the percentage of Ca and P RNI covered by 100 g, reaching 13.06% and 47.78%, respectively. It is noteworthy that Mg has the smallest coefficient of variation (9.64%) among the macro-elements, and its value is lower than that of the other micro-elements. This result indicates that the content of Mg in Chinese walnuts is relatively stable. However, the average elemental Mg concentration in this study was higher than in other regions, exceeding 55% of the data published for the French region [35]. When the Na content was compared to other regions, it was observed that there was a large variance in Na content between regions, reaching up to 5723.5 mg kg^{-1} in the Serbian report [34]. The average content of Na in this study was only 11.5 mg kg$-^{1}$. In terms of recommended nutritional intake, 100 g of WF provides 62.87% of the RNI for Mg and 0.05% of the RNI for Na. A low-Na diet has been found to be advantageous in maintaining good blood pressure and preventing cardiovascular disease [36]. WF could be utilized to make salt-free products due to its low Na level. Among the micro-elements, 100 g of Mn and Cu contain more than 1.5 times the RNI, while Fe and Zn account for 23.59% and 31.71% of the RNI, respectively. Overall, using WF to increase the nutritional content of gluten and gluten-free baked items could be advantageous.

3.3. Amino Acids in Walnut Flour

Amino acids are one of the key components that influence walnut quality and have significant and favorable impacts on the human body. The total amino acid content of WF was found to be 28.28 g 100 g^{-1} in this investigation. Figure 1 shows the amino acid composition data, which shows the existence of 17 amino acids, including seven essential and 10 non-essential amino acids. The most prevalent non-essential amino acids in WF were found to be glutamic acid (Glu) (5.95 g 100 g^{-1}), arginine (Arg) (4.39 g 100 g^{-1}), and aspartic acid (Asp) (2.84 g 100 g^{-1}), accounting for 46.59% of the total amino acids present. Leucine (Leu) (2.07 g 100 g^{-1}) was discovered to be the most abundant necessary amino acid, while methionine (Met) (0.31 g 100 g^{-1}) was found to be the least abundant. The average concentration of the seven essential amino acids tested in WF ranged from 0.31 to 2.07 g 100 g^{-1}. Furthermore, WF also contains a lot of isoleucine (Ile) and phenylalanine (Phe): 1.07 and 1.28 g 100 g^{-1}, respectively. The contents were discovered to be greater than those reported in walnuts by Keun Hee Chung from Korea [37].

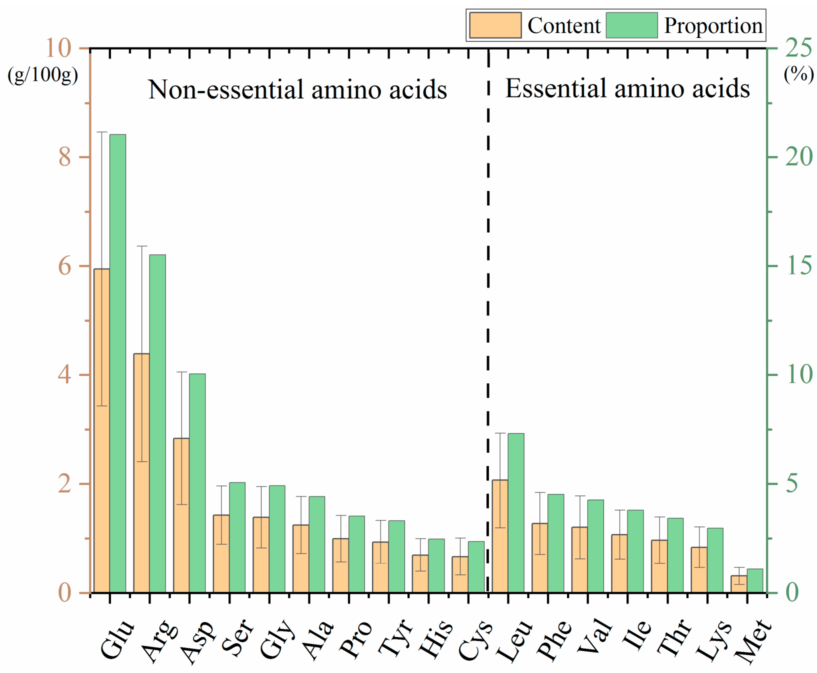

Figure 1. Amino acid profile content and proportions in analyzed walnuts. Note: the yellow bars represent the amino acid content; the green bars represent the proportion of the amino acid in the total amino acid; the dotted lines on the left side are non-essential amino acids, and the dotted lines on the right side are essential amino acids.

The high percentage of Glu identified in the WF samples of this study is consistent with previous research, indicating that Glu is the primary non-essential amino acid present in plant-based foods such as wheat, which has a glutamic acid level of 27.44% [38]. Leu is an important amino acid that belongs to the branched-chain amino acid group and aids in insulin release, which aids in blood sugar regulation. In this study, the percentage of Leu in walnuts reached 7.40%, which is equivalent to cereals, such as wheat [38]. This implies that eating WF may help prevent non-insulin-dependent diabetes. Previous research has shown that lysine (Lys) and threonine (Thr) are the amino acids most limited in grains like wheat [19] owing to their low proportion. Thr, for instance, is present in fewer than 2% of the total. Although their absolute concentration is lower than that of wheat, their total amino acid composition ratio is superior to that of wheat. In particular, the percentage of Thr in WF was 2.36 times higher than in wheat flour [38]. The proportion of Lys in WF was 32.32% greater than in wheat flour. This implies that a small amount of WF in wheat meals is beneficial to amino acid equilibrium.

3.4. Correlation between the Nutritional Qualities of Walnuts

In order to highlight the potential interdependence of nutritional values, we performed a Pearson correlation analysis on the nutritional qualities of 273 walnut samples, as shown in Figure 2 (the correlation heat map only shows r > 0.5, see the Supplementary File (Table S3) for more information). Except for Cys, all amino acids exhibited positive correlations ($p < 0.01$, r > 0.70). Except for Cys, Fe, Na, and tocopherol were all negatively correlated with all amino acids ($p < 0.01$, r > 0.60).

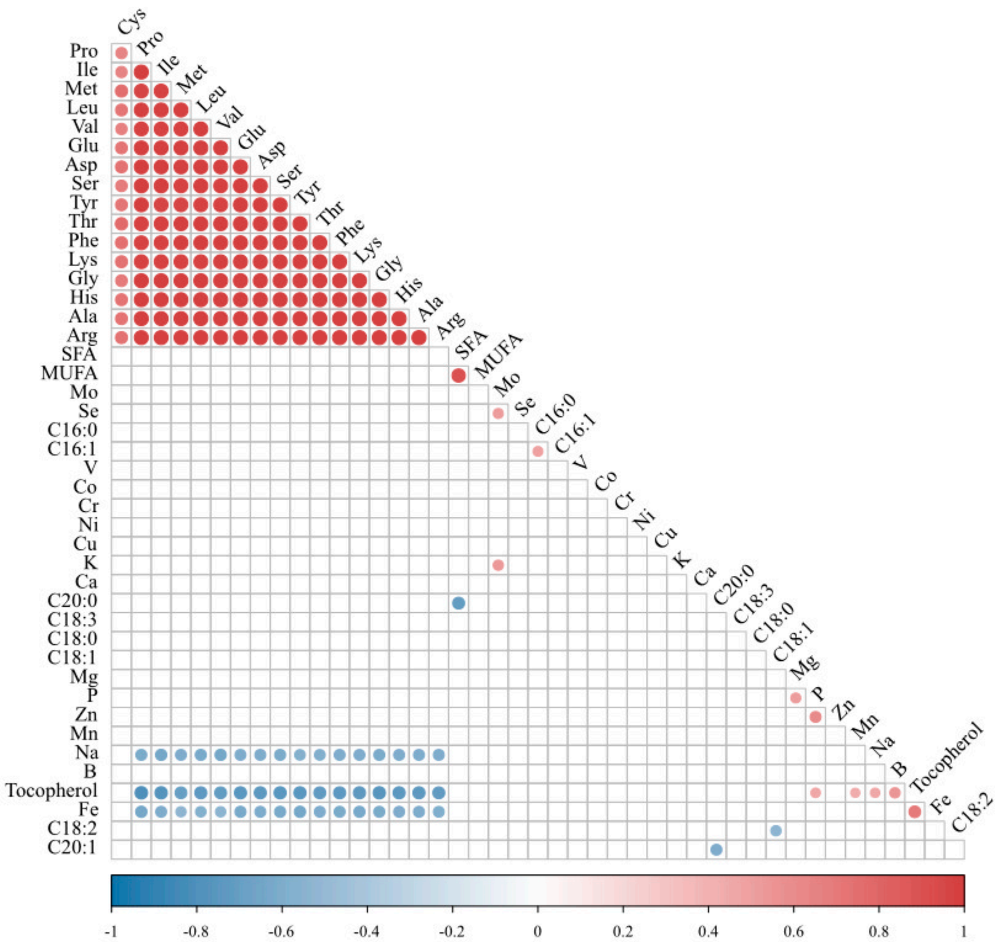

Figure 2. Correlation among the nutritional components of walnuts. Note: Red: positive correlation; Blue: negative correlation. The figure only shows the results with correlation coefficients greater than 0.5, and the specific correlation coefficient results can be found in the Supplementary File (Table S3).

It is becoming increasingly common for studies to report high correlations between amino acids in agricultural products. For example, researchers found positive correlation coefficients between amino acids in soybeans, observing significantly high positive correlations between methionine and Lys ($p < 0.01$, r = 0.70) and Cys ($p < 0.01$, r = 0.57), respectively [39]. Additionally, a strong correlation dependence between Gly, Ser, Thr, Ile, Val, and Pro was discovered in tomatoes ($p < 0.01$, r > 0.87) [40]. These findings highlight the complex mechanisms involved in the synthesis and metabolism of amino acids [41].

Fertilizers are currently utilized in agricultural production to ensure sufficient levels of macro- and micronutrients for optimal fruit growth and development, thereby enhancing yield and quality. Available studies have demonstrated that the application of inorganic fertilizers can increase the content of inorganic elements in crops. After the application of inorganic fertilizers, there was an increase in the content of P, K, Mg, and Cu in the fruits of rapeseed seedlings [42]. The content of K, Ca, Mg, and Na in fennel bulbs increased after treatment with P and K fertilizers [43]. However, there is no conclusive evidence yet on the relationship between the application of inorganic fertilizers and changes in the organic nutrient content in crops. Some studies have found that increasing the use of P fertilizer can affect the levels of amino acids and inorganic elements in goji berries [44]. The current study found a strong negative correlation between Fe and Na and most amino acids, especially Ile in walnuts, with Fe and Na having a correlation coefficient greater than 0.60. Meanwhile, scholars have found that Fe is involved in amino acid biosynthesis in Arabidopsis to fine-tune the production of specific amino acids [45]. As a result, we hypothesize that the observed negative correlation between the mineral elements and amino acids is due to the process of amino acid anabolism. According to Ahmed's study, it was found that the protein and essential amino acid content of sorghum seeds increased significantly ($p < 0.05$) with the addition of micronutrient fertilization [46]. However, further research is still required to determine whether higher levels of amino acid content in walnut can be achieved through the use of inorganic fertilizers.

3.5. Soil Qualities and Their Correlations

Soils store a variety of nutrients required for proper plant growth and development, and soil parameters are interconnected. The average concentrations of mineral elements, pH, and soil organic matter (OM) in soil samples collected from walnut-growing areas in China were analyzed. Fe was the most abundant element in the soil samples analyzed in this study, with levels ranging from 19,717.70 mg kg^{-1} to 41,802.39 mg kg^{-1}, while the K levels ranged from 18,715.00 mg kg^{-1} to 26,423.33 mg kg^{-1}. Mg was found in high concentrations in the soil samples (5663.33 mg kg^{-1} to 22,146.67 mg kg^{-1}). The other 12 micro-elements ranged from 18,071.67 mg kg^{-1} to 0.29 mg kg^{-1} in the soil (detailed data can be found in the Supplementary File, Table S4). In addition, another important factor influencing soil health is the level of organic matter content, which is necessary to ensure nutrient release, biological activity, and the soil's ability to sustain agriculture [47]. In this study, the OM content of the soil ranged from 7.79 g kg^{-1} to 26.79 g kg^{-1}.

Figure 3 depicts a variable correlation analysis of walnut orchard soils. We observed significant ($p < 0.01$) positive correlations between Zn, Cu, Fe, Cd, Co, and Ni, with correlation coefficients above 0.57, suggesting that increasing Ni content in soil may increase the content of most mineral elements. Interestingly, there was a significant antagonistic relationship between Mg-K and Mg-Ba ($p < 0.01$, r > 0.72). Several studies have been conducted to explain the significant correlation between trace elements by focusing not only on the abundance of biological factors and element inhibition but also on trace element fixation or adsorption by soil [48]. The relationship between soil pH, soil OM, and mineral elements exhibited distinct characteristics. Soil pH was found to have a negative correlation with most mineral elements, especially Cu, with a correlation value of 0.65, indicating that more alkaline soils are more prone to exacerbated trace element loss. Soil OM, on the other hand, had significant correlations with Fe, Cd, Ni, Cu, and Zn ($p < 0.01$, r > 0.63). A study of fertilized farmland in China found a weak negative and positive relationship between soil pH and elements [49]. Similarly, in a soil study in southeastern China, a significant relationship between Cu and OM was found ($p < 0.01$, r > 0.60) [50].

3.6. The Impact of Soil on the Nutritional Qualities of Walnuts

Scholars have conducted extensive research on the relationship between soil qualities such as mineral elements, pH and OM, and fruit quality [51,52]. The titratable acidity of kiwifruit fruit was significantly negatively correlated with alkaline hydrolyzed nitrogen content in the soil ($p < 0.01$, r = 0.94). In contrast, a positive correlation (r > 0.5) between fruit organic acid content and soil calcium/potassium (Ca/K) levels was found in a report on apples. In this study, Pearson correlation was employed to investigate the relationship between soil nutrients and soil quality. Regarding correlation coefficients greater than 0.50, a network diagram was created (Figure 4). Overall, the effect of soil micronutrients on fruit quality was strong, while the effect of macronutrients on fruit quality was weak. It is clear that the soil elements Fe, Cu, and Zn have a greater influence on the majority of walnut qualities. Furthermore, all three elements correlated strongly with α-tocopherols, δ-tocopherols, C18:2, Ca, and K in the kernel, especially K in walnuts, with correlation coefficients of greater than 0.60. The Fe in the soils was significantly negatively correlated with both K and α-tocopherols, with correlation coefficients above 0.80. The Cu in the soils was significantly positively correlated with walnut Ca, with a correlation coefficient of 0.82. The Zn in the soils was positively correlated with most elements, particularly C18:2 and γ-tocopherols, with correlation coefficients of 0.66 and 0.57, respectively. In addition, Zn was the only element in the soils that correlated with all four amino acids (Arg, Val, Met, and Phe), with a level of greater than 0.50. This suggests that Zn in the soil has a beneficial effect on the majority of walnut qualities.

A previous study showed that soil OM and pH have a significant impact on fruit quality. In contrast, the findings of this study revealed that the correlation coefficients between OM and walnut quality were all less than 0.50. However, a strong correlation was discovered between soil pH and walnut quality, particularly in terms of Ca and Mn in walnuts, with correlation coefficients of 0.78 and 0.72, respectively. It is important to note that the amount of Mn in walnuts is influenced by a variety of soil conditions. Correlation coefficients greater than 0.50 are found for Zn, K, Mn, and Na and pH. According to the data, soil micro-elements, specifically Fe, Cu, and Zn, have the greatest influence on fruit quality. Furthermore, the pH level of the soil has a significant impact on walnut quality.

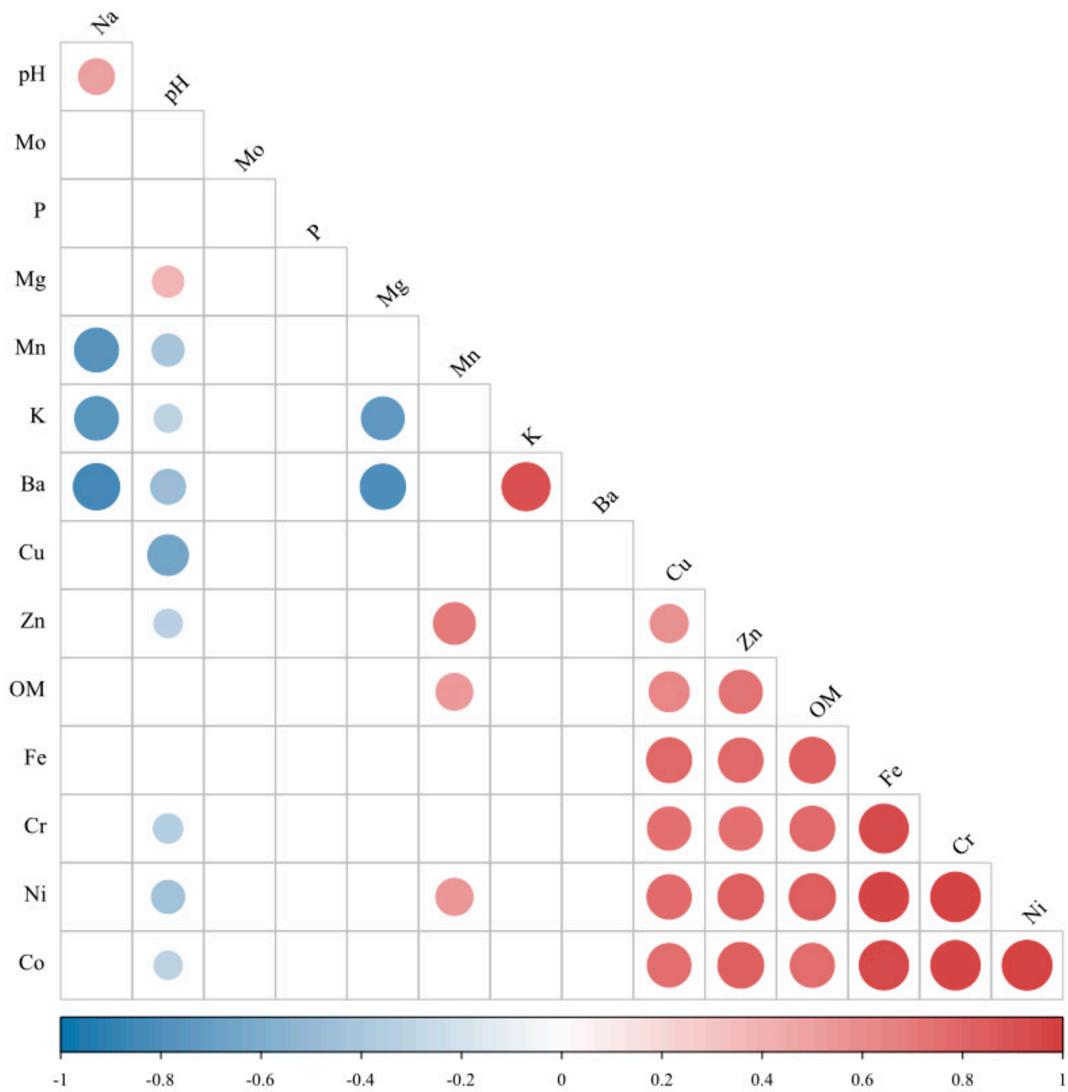

Figure 3. Correlations between soil properties. Note: Red: positive correlation; Blue: negative correlation. The figure only shows the results with correlation coefficients greater than 0.5, and the specific correlation coefficient results can be found in the Supplementary File (Table S5).

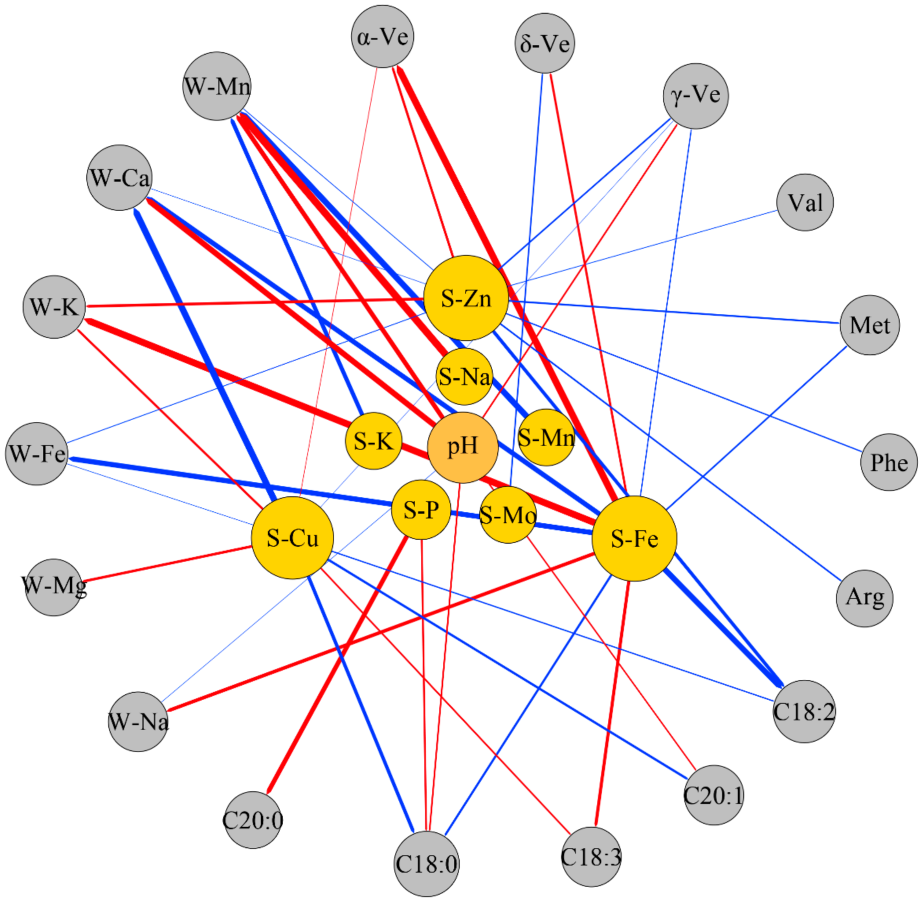

Figure 4. The impact of soil on the nutritional qualities of walnuts. Note: blue and red lines represent positive and negative correlations between nodes, respectively; the thickness of the line is positively correlated with the Pearson correlation coefficient (the figure only shows the results with correlation coefficients greater than 0.5). The size of the circle is related to the number of nodes connected; grey circles represent the nutritional components of walnuts; yellow circles represent soil parameters.

4. Conclusions

This is the first comprehensive report on the quality of English walnuts and the soils in which they are grown in China. The findings of this study revealed a significant amount of C18:2 in Chinese walnuts when compared to previous reports, with a proportion of 62.36%. The predominance of γ-tocopherol (77%) is a key feature that distinguishes walnuts from other nuts. When compared to wheat flour, which is commonly used as a baking ingredient, WF contains more minerals, particularly Mg, with a concentration that is more than seven times higher. In addition, Thr and Lys in walnut have a better total amino acid ratio than in wheat. The content of three trace elements (Fe, Cu, and Zn) in the soil has the greatest influence on the nutritional qualities of walnuts. Furthermore, pH, as a fundamental physical and chemical property of the soil, has a significant impact on the quality of walnuts. Overall, this study not only sheds new light on the quality regulation of walnuts and identifies the potential of walnut flour applications in bakery products, but it also provides data for consumer choice and trade.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14071369/s1, Figure S1: Geographical location of walnut sample collection sites in eight provinces; Table S1:; Information on where soil samples were collected; Table S2: Comparison of the mineral composition in walnut flour from eight provinces.; Table S3: Correlation among the nutritional components of walnuts; Table S4: Soil properties (pH and OM) and content of 12 mineral elements in the soil; Table S5 Correlations between soil properties

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