The effects of cultivar and climate zone on phytochemical components of walnut (*Juglans regia* L.)

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**Abstract**
The diversity of cultivars and variation in phytochemical components pose a great threat to the security of foods that contain walnut. The nutrient values of walnut are stipulated according to phytochemical components, which are driven by internal and external factors associated with fruit growth. The objective of this work was to investigate the effects of cultivar and climate zone on the phytochemical components of walnuts. First, differences and relationships of phytochemical components in eleven walnut cultivars harvested from northeastern China were analyzed. Almost all individual phytochemical components among cultivars showed significant differences (\(p < .05\)). The results were submitted to correspondence analysis followed by matrix transformation and factor analysis. Fatty acids, amino acids, and minerals (Ca, Na, Fe, Cu, P, and Zn) for the identification and discrimination of eleven walnut cultivars were authenticated. Furthermore, based on essential microelements, essential amino acids, unsaturated fatty acids, and antioxidants, the walnut cultivars realized the precision position in food application. Finally, we confirmed the effects of climate zones on the phytochemical components of walnuts, with variations up to 142%. The climatic factors precipitation and temperature influenced the formation and accumulation of monounsaturated fatty acids, tocopherols, and most minerals. The findings will open promising perspectives for oriented cultivation of walnuts and future application in the food industry.

**KEYWORDS**
cclimate zone, correspondence analysis, phytochemical components, variations

1 | **INTRODUCTION**

Walnut is the most widespread tree nut in the world and ranks second behind almonds in tree nut production. According to the Food and Agriculture Organization of the United Nations, more than 60 countries are involved in the production of walnuts, such as China, Iran, the United States, Turkey, France, and Brazil (Abdallah et al., 2015). The cultivation of walnut trees worldwide has encouraged the development of new walnut cultivars, adapted to the environmental conditions of their growing...
area and the preferences of the local markets (Rabadán, Pardo, Pardo-Gimenez, & Alvarez-Ortí, 2018). Recent epidemiologic studies indicated that walnut consumption was effective in reducing the incidence of cardiovascular mortality, coronary heart disease, hypertension, and cancer (Ojeda-Amador, Salvador, Gomez-Alonso, & Fregapane, 2018; Ros, Izquierdo-Pulido, & Sala-Vila, 2018). Walnuts have been long valued for human health, nutritional properties, and also in the food industry due to abundant phytochemical components.

Walnut is a rich source of fatty acids, polyphenols, flavonoids, tocopherols (Ve), essential amino acids, and minerals (Arcan & Yemencioglu, 2009; Chen et al., 2015). In particular, walnut virgin oil with a predominance of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) is high-quality vegetable oil (Cuesta et al., 2017). Among the phytochemicals, polyphenols, flavonoids, and Ve have attracted considerable attention due to their antioxidant, antibacterial, antifungal, anti-inflammatory, anti-aging, anticarcinogenic, and neuroprotective effects (Vu, Vo, Coggeshall, & Lin, 2018). Scientists have paid more and more attention to internal relationships and formation mechanisms of phytochemical components in walnuts. Some significant correlations regarding the maturation of walnuts among fatty acids (Li et al., 2017) and amino acids (Rao, Sui, & Zhang, 2016) were reported. These previous studies only involved a single nutritional quality or specific components. Apparently, comprehensive information concerning the correlations of more phytochemical components in walnuts remains to be further mined. Cultivar variations of phytochemical components have been reported for the most important commercial nuts (Rabadán, Alvarez-Ortí, & Pardo, 2019). For walnuts, significant differences were noted in many reports with respect to the levels of fatty acids (Li et al., 2017), minerals (Trandafir, Cosmulescu, Botu, & Nour, 2016), and polyphenols (Vu et al., 2018). The component variability among cultivars in different planting areas was especially apparent. In a farm of Poland, eleven walnut cultivars exhibited a wide content of polyphenols from 8.2 to 20.7 mg/g (Pycia, Kapusta, Jaworska, & Jankowska, 2018), and there was 51.54% variation in the phenolics in black walnuts from American plantations (Vu et al., 2018). However, in walnut plantations in Romania, no significant differences in total phenolics and flavonoids were found, while the contents of some minerals were apparently different (Trandafir et al., 2016). Different degrees of variations in fatty acids and Ve were also reported in main walnut plantations (Abdallah et al., 2015; Cuesta et al., 2017; Pereira et al., 2008; Pogetti, Feruia, Chiaba, Testolin, & Baldini, 2018; Tapia et al., 2013). Moreover, there are many walnut cultivars, which have in the past couple of years engrossed the agricultural production market. Such an expansive genetic resource causes confusion in walnut production and consumption. Due to a lack of knowledge of specific components and characteristics, second-rate vendors and degradation of quality due to extreme climatic changes have seriously endangered the security of walnut foods and long-term development. In this study, one of our main aims was to reveal variations and relationships of phytochemical components among walnut cultivars and to determine the precise position of different cultivars in terms of nutrient and commercial values.

In addition to cultivar, climatic conditions in planting areas have direct effects on phytochemical components. Rabadán et al. (2019) revealed that minerals (Fe) and protein in walnuts were determined by the change of weather conditions. By analyzing 29 nuts (including walnuts) collected from the southeast of Spain, the variances caused by crop year conditions in the content of fatty acids, polyphenols, Ve, and essential minerals were greater than those related to cultivars (Rabadan et al., 2018). As a result, more studies should focus on the effect on walnuts for a wider analysis of specific variables related to environmental factors. Specific climatic factors play an important role in the formation and accumulation of phytochemical components during the growth stage of walnut. Lower minimum temperatures could promote the synthesis of unsaturated fatty acids (Fuentealba et al., 2017; Rudell et al., 2011), while rainfall directly influences the contents of phenolic compounds during kernel-growing seasons (Lynch, Koppel, & Reid, 2016). Drought and low rainfall also led to a surge in the Ve content of walnuts from the high Atlas Mountains (Kodad, Estopanan, Juan, Company, & Sindic, 2016). We found that it was difficult to reach consistent conclusions from these reports. Excluding the influence of human factors and cultivar, interference of many environmental factors and differences in planting areas were the main reasons. The climate zone determines all environmental factors. Significant variation of inner components exists naturally in plant fruits due to environmental factors that occur during fruit growth, such as precipitation, temperature, latitude, drought, and radiation (Poggetti et al., 2018). These factors can be ultimately attributed to the differences of the climatic zones in the planting areas. Studies on phytochemical components from the level of climate zone could effectively prevent the influence of regional and environmental factors. Koppen climate classification is widely used in the field of geography because of its strict climate index and clear boundaries (Zeroual, Assani, Meddi, & Alkama, 2019). Therefore, another aim of this study was to explore the climate zone effect on phytochemical components of walnuts and to determine the effect of specific climatic factors.

## 2 MATERIALS AND METHODS

### 2.1 Chemicals and reagents

HPLC grade of iso-octane, n-hexane, and isopropanol and analytical grade of all reagents and solvents were purchased from Merck. The standards (gallic acid, catechin, caffeic

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**MATERIALS AND METHODS**

**Chemicals and reagents**

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acid, trolox, campesterol, stigmasterol, α-Ve, β-Ve, δ-Ve, γ-Ve, ascorbic acid, and dehydroascorbic acid) were from Sigma Chemical Company. Nitric Acid, hydrogen peroxide, sodium carbonate, sodium nitrite, hydrochloric acid, aluminum nitrate, and light petroleum ether (analytical grade, b. p. 60–90°C) were purchased from Sinopharm Chemical Reagent Limited Corporation.

### 2.2 Plant material and sample treatment

The eleven walnut cultivars (Liao 1, Liao 4, Liao 5, Liao 6, Liao 7, Liao 10, Li 1, Li 2, Liaoruifeng, Hanfeng, and 10901) were collected in 2017 from Liaoning Province, northeastern China. Liaoning province is the northern boundary of walnut cultivation in China. The climate here is warm continental climate with the annual mean temperature of 7–11°C, frost-free period of over 150 days, annual precipitation of 550 mm on average, and sunshine of 2,100–2,600 hr/year. All samples (3 kg walnuts) came from 10- to 15-year-old trees of local orchard during mature stage. At every collection place, we randomly collected 10 walnuts with the same degree of maturity from the same walnut tree. Green skin was removed mechanically, and the walnut shell was manually cracked. Finally, the walnut kernels were stored at −20°C until analysis.

### 2.3 Fatty acid compositions

Soxhlet was employed to extract walnut oil samples. Fatty acid methyl esters (FAME) from oil samples were obtained by alkaline treatment (2.0 M KOH in methanol). The FAMEs were analyzed using a 7890A Gas Chromatography equipped with a flame ionization detector (Agilent Technologies, Santa Clara, CA, USA). The GC analysis was carried out using an HP-INNOWax fused silica capillary column (30 m × 0.25 mm × 0.25 μm). The detailed operation conditions were as follows: He as carrier gas, the injector temperature at 220°C, split ratio 1:100, and detector temperature 275°C. The column was held for 1 min at 140°C and then programmed at 4°C/min to 250°C. The external standard calibration method was used to quantify the samples. C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, and C20:1 represented palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, and eicosenoic acid, respectively.

### 2.4 Amino acids

The amino acids were analyzed by L-8900 Amino Acid Analyzer (Hitachi High Technologies Corporation) after precolumn derivatization with O-phthaldialdehyde (OPA). The 0.1 g samples were hydrolyzed with 10 ml HCl (6 M) at 105°C for 24 hr under nitrogen atmosphere.

### 2.5 The content of antioxidants

The total polyphenol and flavonoid contents were measured at 510 and 765 nm in an Optimize Pop UV/Vis Spectrophotometer (PerkinElmer), respectively. Details about the analysis procedure were described in our previous work (Han et al., 2019).

The Ve were quantified according to the external standard curves. 0.3 g of oil was dissolved in 5 ml of n-hexane. After vortex for 30 s, the solution was filtered through a 0.45-μm nylon filter and was determined by high-performance liquid chromatography (HPLC) (Agilent Technologies, Palo Alto, CA, USA). The mobile phase was the mixture of isopropanol and n-hexane (2:98, v/v), and the separation column was a Prodigy ODS-2 Column (2 × 150 mm × 4.6 mm, 5 μm). The excitation wavelength and emission wavelength through fluorescence detector were set at 295 nm and 333 nm, respectively.

### 2.6 Mineral composition

Each 0.2 g walnut sample was accurately weighed into a 50-ml polytetrafluoroethylene (PTFE) tube. Then, 3 ml of 65% HNO3 (w/w) solution and 2 ml of 30% H2O2 (w/w) solution were added to the sample in PTFE tube. Next, the PTFE tube was digested in a MARS 5 Microwave System (CEM). Potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P), sodium (Na), manganese (Mn), copper (Cu), iron (Fe), and zinc (Zn) were quantified using ICP-OES (Thermo, Japan); boron (B), barium (Ba), cobalt (Co), molybdenum (Mo), nickel (Ni), selenium (Se), titanium (Ti), thallium (Tl), and vanadium (V) were determined by NexION 300D ICP-MS.

### 2.7 Correspondence analysis

Correspondence analysis (CA) is the weighted principal component analysis (WPCA) of data from contingency tables. The basic idea of this approach is to present the proportional structure between the rows and the columns of contingency tables in a lower dimension in the form of points (Lei, Li, Cheng, Wang, & Meng, 2018). Correspondence analysis aims to project the vector points of high-dimensional space to low-dimensional space by matrix transformation and factor analysis, and then show
the different levels of association of multiclassification variables on a two-dimensional graph. The classification points of variables in the quadrant are related to each other. The closer the point-to-point distance, the higher the association. In our research, correspondence analysis was developed to display the different cultivars and chemical components in a graph with the same coordinate axis (factor axis) in order to reveal the relationships between cultivars and phytochemical components. The corresponding eigenvectors of samples and components were calculated by the system according to the two larger eigenvalues. A diagram of the correspondence analysis between cultivars and phytochemical components was obtained with the eigenvectors.

2.8 Koppen climate classification

Koppen's classification is based solely on the mean values of precipitation and temperature. The Koppen climate zones with the division of climate into five zones are designated using a three-letter scheme (Zeroual et al., 2019). The five climatic zones are tropical (A), dry (B), temperate (C), continental (D), and polar (E) climate. The first of these letters, in upper case, indicates the vegetation group in the climate zone defined based on temperature and precipitation. The second letter, in lower case, reflects the distribution of precipitation at the annual scale. The last letter reflects seasonal temperature variations.

The details of the Koppen climate classification can be seen in Table 1. To this day, Koppen climate classification is widely used in many fields because of its scientific basis and normative division, the latest of which was presented in detail by Kottek, Grieser, Beck, Rudolf, and Rubel (2006).

We collected typical reports (14 previous literature studies and our results) since 2000 on many phytochemical components of different walnut cultivars (Juglans regia L.). The details can be seen in Table S1. Based on Koppen climate classification, the planting areas from these reports were divided into specific climatic zones. Walnuts are basically grown in C and D climate zones, with a few areas in the B climate zone, and none in the A or polar E climate zone. According to specific climatic zones from reports, the level and coefficient of variation (CV) of every investigated component (MUFA, PUFA, VE, Mg, K, Na, Fe, Mn, Cu, Zn, Ca, and P) were classified into three categories in Table S2 to provide a comparative study.

2.9 Statistical analysis

The results were shown as mean ± standard deviation. The statistical differences of the measured data were calculated by analysis of variance (ANOVA). The t test in SPSS, version 19.0 (IBM), was further used to analyze the statistical significance (p < .05) between different regions. The correspondence analysis and multiple correspondence analysis

| Main Climate | Temperature °C | Precipitation | Main Climate | Temperature °C |
|--------------|----------------|---------------|--------------|----------------|
| A Tropical   | >18            | f ≥60 mm      | B Arid       | /              |
|              | /              | m <60 mm      |              | w <50% (precipitation/threshold) | h |
|              | s <60 mm      | >4%           |              | k >50% (precipitation/threshold) | <8 |
| C Temperate  | 0–18           | s max > 3 min | D Continental | w max > 10 min |
|              | >10            |               |              | f even |
| E Polar      | <0             | T /           |              | c min < 0 |
|              | <10            | F /           |              | 1 month > 10 |

Note: The C (temperate) and D (continental) climate have the same subclimates of “s, w, f” and “a, b, c.” “/” mean no information available. All climate zones include “Af, Am, Aw, Bwh, Bsk, Bsk, Cs, Csb, Csw, Cwb, Cwc, Cfs, Cfb, Cfc, Dsa, Dsb, Dsc, Dwa, Dwb, Dwc, Dfa, Dfb, Dfc, ET, EF.”
between different phytochemical components of walnuts were analyzed by R language (version 3.5.2).

3 | RESULTS AND DISCUSSION

3.1 | The differences in phytochemical components among the various walnut cultivars

Data on the phytochemical components of eleven walnut cultivars are shown in Table 2a and b. Significant differences among cultivars were found for almost all individual phytochemical components.

The contents of major fatty acids in walnut oils are important for evaluating the economic and nutritional values. Palmitic acids and stearic acids were the main saturated fatty acids found, agreeing with previous studies (Li et al., 2017; Rabadan et al., 2018). The walnut oils had high PUFA contents, which are beneficial fatty acids for human health. The PUFA content accounted for more than 73.00% of fatty acids in Liao 5 and only 58.23% in Liao 7. Among common foods, walnuts are one of the most important sources of total polyphenols, with a reported content of up to 25 mg/g (Sanchez-Gonzalez, Ciudad, Noe, & Izquierdo-Pulado, 2017). The mean content of polyphenols in our work was higher, that is, 29.30 mg/g. The content in Liao 4 was highest, that is, 45.02 mg/g, compared with only 13.34 mg/g in Liao 10. Similar to the polyphenols, the flavonoid content also showed a wide range of 5.53–22.30 mg/g among cultivars. The highest polyphenol and flavonoid contents were found in Liao 4. Compared with the polyphenols and flavonoids, the difference in the Ve content among cultivars was relatively low. High values of total Ve detected were 0.30 mg/kg, in which γ-Ve and δ-Ve were present in major quantities.

The United States Food and Drug Administration (FDA) approved that walnut protein including a variety of amino acids can provide several nutritional benefits and can affect sensory properties of foods (Robbins, Shin, Shewfelt, Eitenmiller, & Pegg, 2011; Holscher et al., 2018). In our study, Glu (3.79 g/100 g, 21%) and Arg (2.8 g/100 g, 15.5%) were predominant amino acids. For cultivar Liaoruifeng, 11 of 17 analyzed amino acids (Asp, Ser, Glu, Gly, Ala, Tyr, His, Arg, Thr, Leu, and Phe) showed the highest values. For minerals, the average values for P, K, Mg, and Ca (major minerals) were 3,610.8, 2,727.5, 1,592.8, and 1,427.2 mg/kg, respectively. In agreement with previous study, the contents of K and P were the highest among all minerals (Gharibzahedi et al., 2011). The average values for Mn, Fe, Zn, Cu, and Na (minor minerals) were 120.0, 51.1, 32.1, 15.4, and 8.8 mg/kg, respectively. Some minor minerals were less reported such as B, V, Ti, Co, Ni, Se, Mo, and Ba, which are strongly associated with an improved health status. The average values were analyzed and are shown in Table 2a and b.

3.2 | The inherent relationships of phytochemical components among the various walnut cultivars

In addition to analyzing the differences, we also investigated the inherent relationships of phytochemical components among the various cultivars. As shown in Figure 1, extremely high correlations among amino acids were found in our analyzed walnuts. Due to the metabolic complexity of amino acids, the synthesis pathway and regulation mechanism have always been the focus of plant physiology research (Song et al., 2017). A high correlation is the embodiment of intricate mechanisms in the synthesis and metabolic processes of amino acids. However, there were no significant correlations (p > .05) between minerals and amino acids. The minerals play crucial roles in the activity of enzymes that act as catalysts and antioxidants becoming determinant in the study of the nutritional value of walnuts (Gharibzahed et al., 2011). Nevertheless, surprisingly, mineral elements were also rarely associated with other phytochemical components in our study. On the contrary, the relationships between antioxidants (polyphenolics, flavonoids, and Ve) and antioxidant activity have been studied extensively in previous research (Rocchetti, Chiodelli, Giuberti, & Lucini, 2018; Santos et al., 2018). We found that the content of polyphenolics was positively correlated with total flavonoids (r = .91, p < .01), which concurred with the relationship (r = .99, p < .01) in Juglans sigillata Dode reported by Shi, Zhang, Li, and Pan (2018). It was also reported that the level of flavonoids decreased with a decline in the polyphenol content during the rapid enlargement of fruit (Shi et al., 2018). Among the four Ve, although α-Ve was strongly correlated with β-Ve (r = .96, p < .05), γ-Ve (with the highest content) showed no significant correlation with the other Ve (p > .05). For fatty acids, C18:2 had significantly positive correlations with C16:0 (r = .65, p < .05) and C18:3 (r = .96, p < .05). Interestingly, there were no significant negative correlations among all components, while Li et al. (2017) found that some significantly negative correlation with C18:1-C18:2 (r = .74, p < .05) and C18:1-C18:3 (r = .50, p < .05) existed in 37 walnut cultivars planted in Shanxi Province in China. Thus, the different performance might be due to the various cultivars and planting areas.

3.3 | The effect of walnut cultivars on phytochemical components

Traditional variance analysis is limited to compare component levels from more angles, and it is difficult to entirely reflect the cultivar effects on these components. Correspondence analysis is a procedure for exploring the relationships among two or more sets of variables. This study examined the results...
TABLE 2  (a) The contents of fatty acids (%) and amino acids (g/100 g) of eleven walnut cultivars from northeastern China. (b) The contents of minerals (mg/kg), flavonoids (mg/g), polyphenols (mg/g), and Ve (mg/100 g) of eleven walnut cultivars from northeastern China

| Component | Liao 1 | Liao 4 | Liao 5 | Liao 6 | Liao 7 | Liao 10 | Li 1 | Li 2 | Liaoruifeng | Hanfeng |
|-----------|--------|--------|--------|--------|--------|--------|------|-----|-------------|--------|
| C16:0     | 5.61 ± 0.05d | 6.05 ± 0.04bc | 5.58 ± 0.02e | 6.13 ± 0.02d | 5.54 ± 0.04d | 5.55 ± 0.004d | 5.52 ± 0.04d | 5.44 ± 0.04d | 6.21 ± 0.03a | 5.36 ± 0.03e |
| C16:1     | 0 ± 0e | 0.04 ± 0de | 0 ± 0e | 0.02 ± 0.04de | 0.04 ± 0.0b | 0.04 ± 0.05e | 0.02 ± 0.0be | 0.04 ± 0.05e | 0.06 ± 0e | 0.06 ± 0e |
| C18:0     | 3.72 ± 0.04a | 3.38 ± 0.05c | 3.67 ± 0.01b | 2.99 ± 0.06c | 3.64 ± 0.06b | 3.18 ± 0.01b | 2.99 ± 0.06c | 3.13 ± 0.03c | 3.11 ± 0.03b | 3.00 ± 0.01b |
| C18:1     | 22.64 ± 0.01g | 23.29 ± 0.01f | 17.12 ± 0.02k | 19.63 ± 0.11b | 33.49 ± 0.0a | 31.36 ± 0.01b | 25.77 ± 0.01a | 24.46 ± 0.02c | 23.57 ± 0.02b | 24.26 ± 0.01c |
| C18:2     | 58.58 ± 0.08e | 57.43 ± 0.04g | 64.07 ± 0.01a | 61.15 ± 0.10b | 50.72 ± 0.01d | 53.37 ± 0.04g | 57.81 ± 0.01f | 57.43 ± 0.04g | 63.55 ± 0.03b | 63.47 ± 0.03b |
| C18:3     | 9.13 ± 0.01d | 9.53 ± 0.03b | 9.28 ± 0.01c | 9.86 ± 0.02d | 8.6 ± 0.01d | 8.6 ± 0.02d | 8.6 ± 0.01d | 8.6 ± 0.02d | 8.6 ± 0.01d | 8.6 ± 0.02d |
| C20:0     | 0.1 ± 0b | 0.09 ± 0b | 0.09 ± 0c | 0 ± 0h | 0.08 ± 0e | 0.11 ± 0a | 0.09 ± 0f | 0.09 ± 0d | 0.09 ± 0d | 0.07 ± 0g |
| C20:1     | 0.22 ± 0a | 0.15 ± 0.11b | 0.2 ± 0ab | 0.18 ± 0ab | 0.24 ± 0.1a | 0.2 ± 0ab | 0.22 ± 0a | 0.22 ± 0a | 0.19 ± 0ab | 0.18 ± 0a |

(Continues)
| Component | Liao 1     | Liao 4     | Liao 5     | Liao 6     | Liao 7     | Liao 10    | Li 1      | Li 2      | Liaorufeng | Hanfeng   | 10,901 |
|-----------|------------|------------|------------|------------|------------|------------|-----------|-----------|-------------|-----------|--------|
| Ti        | 29.46 ± 0.77ab | 28.69 ± 0.65abc | 28.43 ± 0.80bc | 25.37 ± 1.20de | 23.97 ± 2.23e | 25.83 ± 0.79ab | 26.93 ± 0.21cd | 24.32 ± 0.48c | 30.38 ± 1.26a | 22.13 ± 0.70f | 27.46 ± 0.48c |
| Mn        | 101.84 ± 2.42c | 149.94 ± 11.71a | 89.07 ± 1.52d | 85.37 ± 3.62d | 140.7 ± 10.83a | 104.82 ± 3.15c | 72.019 ± 3.14e | 68.85 ± 1.19e | 115.33 ± 6.96b | 51.80 ± 2.98f | 119.9 ± 8.21b |
| Fe        | 48.10 ± 1.25efg | 47.53 ± 0.72fg | 48.82 ± 1.38g | 53.63 ± 3.54cd | 52.01 ± 4.44cd | 55.71 ± 2.71c | 60.08 ± 1.78b | 53.6 ± 0.96cd | 66.09 ± 2.90a | 51.42 ± 1.66de | 51.1 ± 0.5def |
| Co        | 0.16 ± 0c | 0.18 ± 0b | 0.20 ± 0a | 0.09 ± 0g | 0.14 ± 0.01d | 0.16 ± 0.01c | 0.08 ± 0.01g | 0.07 ± 0h | 0.11 ± 0f | 0.13 ± 0.01e | 0.12 ± 0f |
| Ni        | 2.9 ± 0.14b | 2.61 ± 0.09c | 2.61 ± 0.04c | 2.35 ± 0.10de | 2.17 ± 0.21ef | 2.77 ± 0.11bc | 3.60 ± 0.10a | 2.70 ± 0.10c | 2.77 ± 0.06bc | 2.14 ± 0.11f | 2.41 ± 0.03d |
| Cu        | 13.16 ± 0.32ef | 14.05 ± 0.25cd | 12.85 ± 0.27f | 13.59 ± 0.18def | 13.13 ± 1.00ef | 14.69 ± 0.48bc | 17.39 ± 0.35a | 14.0 ± 0.13cd | 17.83 ± 0.35a | 13.79 ± 0.56de | 15.42 ± 0.20b |
| Zn        | 30.74 ± 1.1ld | 26.54 ± 0.64f | 27.29 ± 2.12f | 27.86 ± 0.94f | 31.06 ± 2.47cd | 34.37 ± 1.09ab | 32.4 ± 0.31bcd | 26.29 ± 0.45f | 35.08 ± 0.60a | 33.34 ± 2.11ab | 32.1 ± 0.8bcd |
| Se        | 0.03 ± 0e | 0.03 ± 0f | 0.04 ± 0d | 0.04 ± 0b | 0.04 ± 0d | 0.04 ± 0b | 0.04 ± 0d | 0.03 ± 0.01f | 0.04 ± 0c | 0.05 ± 0a | 0.02 ± 0.01g |
| Mo        | 0.08 ± 0f | 0.07 ± 0f | 0.11 ± 0d | 0.15 ± 0.01b | 0.11 ± 0.01d | 0.10 ± 0e | 0.14 ± 0b | 0.14 ± 0.01b | 0.18 ± 0.01a | 0.17 ± 0a | 0.13 ± 0c |
| Ba        | 3.02 ± 0.10f | 3.30 ± 0.09f | 3.01 ± 0.13f | 4.38 ± 0.15d | 4.56 ± 0.31d | 3.80 ± 0.03e | 5.41 ± 0.18b | 4.00 ± 0.07c | 6.73 ± 0.22a | 3.27 ± 0.11f | 5.10 ± 0.22c |
| Ti        | 0.003 ± 0f | 0.005 ± 0de | 0.005 ± 0de | 0.005 ± 0de | 0.002 ± 0e | 0.007 ± 0b | 0.006 ± 0cd | 0.005 ± 0e | 0.006 ± 0e | 0.012 ± 0a | 0.006 ± 0cd |
| Flavonoid | 15.96 ± 0.83cd | 22.3 ± 5.19a | 18.34 ± 0.94bc | 8.53 ± 0.45gh | 17.6 ± 1.39cd | 5.53 ± 1.07h | 9.27 ± 0.48gh | 14.0 ± 1.58de | 10.29 ± 1.07f | 19.84 ± 1.84ab | 12.32 ± 2.2ef |
| Polyphenol| 36.06 ± 1.71bc | 45.02 ± 7.62a | 41.29 ± 2.04ab | 20.08 ± 1.64g | 31.12 ± 5.57cd | 13.34 ± 2.52eh | 23.4 ± 0.78efg | 30.9 ± 3.34cd | 22.84 ± 3.91fg | 30.0 ± 4.97cd | 28.2 ± 1.7def |
| α-Ve      | 2.00 ± 0.01a | 2.05 ± 0.06a | 2.07 ± 0.07a | 1.79 ± 0.02b | 2.13 ± 0.09a | 0.91 ± 0.17e | 1.1 ± 0.06d | 1.17 ± 0.03d | 1.03 ± 0.03de | 1.31 ± 0.07c | 1.03 ± 0.01de |
| β-Ve      | 1.53 ± 0.01a | 0.46 ± 0.01b | 0.43 ± 0.01b | 0.47 ± 0.02ab | 0.48 ± 0.02ab | 0.14 ± 0.03cd | 0.19 ± 0c | 0.12 ± 0.06d | 0.13 ± 0.05cd | 0.16 ± 0.06cd | 0.14 ± 0.0cd |
| γ-Ve      | 24.72 ± 0.70bc | 26.13 ± 0.47ab | 21.67 ± 1.09g | 22.34 ± 1.36ef | 25.19 ± 1.64bc | 22.22 ± 1.28ef | 23.85 ± 1.48cd | 20.5 ± 0.28g | 25.71 ± 0.74ab | 27.01 ± 0.8a | 23.09 ± 0.0ef |
| δ-Ve      | 5.19 ± 0.17b | 4.7 ± 0.08c | 4.53 ± 0.24cd | 3.54 ± 0.18e | 4.29 ± 0.27d | 3.5 ± 0.05e  | 3.54 ± 0.09e | 3.22 ± 0.03f | 5.47 ± 0.15a | 4.6 ± 0.16c | 5.3 ± 0.05ab |
of correspondence analysis to clarify the relationships between cultivars and phytochemical components. The information is shown by the first two axes in Figure 2. The closer the distance between two points, the closer the relationship between them. A strong association between the cultivars (Liao 1, Liao 5, Liao 7, Liao 10, and Li 2) and fatty acids was observed in the second and third quadrants. The strong associations indicated that these cultivars had high similarity in the contents of fatty acids. The contents of fatty acids have been reported in species determination of pine nuts (Mikkelsen, Jessen, & Ballin, 2014). For walnuts, fatty acids especially C18:1 have been adopted to distinguish and classify walnut cultivars in many reports (Li et al., 2017; Unver, Sakar, & Sulusoglu, 2016). In our study, only Liao 4 cultivar (located in the first quadrant) showed a strong association with C18:1, which played an important role in distinguishing Liao 4. In addition to C18:1, δ-Ve, Pro, Ile, and Leu were strongly associated with Liao 4. In the fourth quadrant, Liao 6, 10,901, and Liaoruifeng were strongly correlated with most amino acids, which showed that these cultivars had similarity in the contents of amino acids.

In additions, for minerals, some of these around the periphery of cultivars such as Ca, Na, Fe, Cu, P, and Zn showed moderate associations with cultivars. By comparison, weak associations were observed between eleven analyzed cultivars and Ti, Mo, Ba, Mn, B, Co, K, and Mn. This meant that those
elements were not that useful in determining the analyzed walnut cultivars. In addition, some antioxidants (polyphenolics, flavonoids, α-Ve, and β-Ve) were also weakly linked to the walnut cultivars. Studies have shown that the heritability of a half-sib family (the same male or female parent) had a significant effect on phenotypic traits (height, diameter, etc.) of the offspring in a black walnut (*Juglans nigra* L.) progeny test (Zhao, Zhang, & Woeste, 2013). In our study, for phytochemical components, the other 10 cultivars revolved in a tight circle, while the Hanfeng cultivar appeared distinct with no any significant linkages with occurrences of all components. The female parents of the 10 cultivars are from the peasant walnut family, in which all have a similar source from Xinjiang paper skin walnuts of China. The Hanfeng cultivar was sourced from *Juglans cordiformis* in Japan. Compared with 10 other cultivars, the absolute difference in female parents might be the key factors for the uniqueness of components in the Hanfeng cultivar. More studies about the importance of parental origin in terms of phytochemical components need to be conducted in future work.

### 3.4 The application positioning of walnut cultivars

In order to determine the cultivars’ superiorities in the food industry, we chose the four specific nutritive components of benefit to dietary health (essential microelements, essential amino acids, unsaturated fatty acids, and antioxidants) to evaluate walnut cultivars by multiple correspondence analysis.

Before the analysis, each specific component was divided into three categories through the method of k-means clustering: "high, middle, and low" levels. Compared with the effects of cultivars on all phytochemical components, we obtained a clearer guideline for specific components (Figure 3). Liao 1, Liao 6, and Liaoruifeng were classified as an independent “group A” on the horizontal axis, which appeared to be associated with a high content of essential amino acids. Walnut milk is one of the high-quality plant protein products that include 8 human essential amino acids, and is widely popular among consumers. Group A was divided into “high-protein” cultivars with potential suitability for the development and processing of high-protein products. As a counterpart, another association was found between walnut group B (Liao 4, Liao 5, and Liao 7) and low content of essential amino acids. However, group B showed a strong association with a high content of flavonoids and polyphenols. Flavonoids and polyphenols are the most important secondary metabolites of plants with multiple biological activities, especially in improving self-protection and shelf life (Poggetti et al., 2018). Therefore, the cultivars in group B were favorable for the development of walnut oil products, with a competitive advantage in functional activity and the extension of shelf life. Li 2 and Hanfeng (group C) were closely correlated with high levels of PUFA and MUFA. PUFA and MUFA especially so-called ω-3 and ω-6 fatty acids showed a protective effect.
against coronary heart disease (Persic, Mikulic-Petkovsek, Slatnar, Solar, & Veberic, 2018). The cultivars in group C were suitable for the development of high-quality walnut oil. Compared to other groups, Li 1, 10,901, and Liao 10 (group D) fully reflected the middle-of-the-road properties, which were associated with the moderate content of most components, such as polyphenols, VE, Val, Phe, Thr, Ile, Fe, and Cu. These cultivars could be adopted for the supply of materials, ingredients, and additives for walnut products (cake, biscuit, and cheese).

3.5 The effects of climatic zones on the phytochemical components of walnuts

Based on Koppen climate classification, the contents of investigated phytochemical components in climate zones are shown in Figure 4 and Figure S1. For fatty acids, the planting areas of walnuts covered three main climatic zones (9 subclimatic zones). The cultivar differences in MUFA content among climate zones were apparent. The CVs among 22 walnut cultivars reached 26% in a temperate climate and 20% in a continental climate. By comparison, the content of PUFA displayed little variation with CVs <6%. It has been reported that precipitation is crucial to the growth of vegetation with climatic sensitivity and the formation of plant nutrients (Du et al., 2019; Meier & Leuschner, 2014). Stearic acid in soybean oil (one type of MUFA) was even found to have a quadratic relationship \( r = .64–.75 \) with total precipitation (Juan, Xinmei, Thelen, & Robertson, 2009). In this work, annual precipitation in the dry climate was found to have a direct effect on the MUFA of walnuts. The MUFA contents in the Bsk climate (annual precipitation/threshold > 50%) were apparently higher than those in the Bwk climate (annual precipitation/threshold < 50%). Besides, the seasonal distribution of precipitation was an important factor for MUFA. The MUFA content in the Dwa climate (monthly mean precipitation: max > 10 min) was apparently higher than that in the Dfa climate. In the Dwa climate, precipitation was...
concentrated in summer and lower in winter, while rainfall was evenly distributed throughout the year with the characteristic of rain-heat inconsistency in the Dfa climate. The same phenomenon was also found in the Csa climate (monthly mean precipitation: max > 3 min) and Cfa climate. Thus, higher precipitation during the walnut growing season was more beneficial to the formation of MUFA.

The planting areas associated with minerals covered three main climatic zones (6 subclimatic zones). Lavedrine, Ravel, Villet, Ducros, and Alary (2000) pointed out that the Na content of “Hartley” walnut cultivar planted in California (Csa climate zone) was higher than that in California (Csa climate zone), and the K level showed the opposite pattern. Based on other previous results, we further found that the levels of Na in the Csa climate were significantly higher than those in other climate zones ($p < .5$). However, in the continental climate, there were low contents of Na and K in walnuts, while Cu, Zn, and Mn had a relatively high content. The CV values from the above-mentioned minerals ranged from 21% to 142% (shown in Figure 4 and Figure S1). The results fully revealed the strong effect of climate zones on minerals. Correspondingly, most mineral elements showed obviously low contents in the dry climate, especially Mn, Cu, Na, K, Ca, and Mg. The performance demonstrated that adequate precipitation might be an essential factor for the accumulation of minerals in walnuts. For Ve, only temperate and continental climatic zones (4 subclimate zones) were mentioned in previous reports. Generally, the level of Ve in continental climate was higher than that in the temperate climate. Notably, for the continental climate, the Ve content was significantly low in the Dfb climate. It has been reported that light and temperature influence the synthesis and degradation of Ve in spinach and tomato (Spicher et al., 2017; Syamila, Gedi, Briars, Ayed, & Gray, 2019). Compared to the Dfa or Dwa climate, lower annual temperature might result in a significant decrease of Ve contents in the Dfb climate.

**CONCLUSION**

The present study investigated the levels of 49 phytochemical components in walnuts and their influential factors (cultivar and climate zone). Comprehensive information concerning the variances and correlations of phytochemical components in different walnut cultivars was analyzed. The study of the effect of the climatic zone on phytochemical components based on Koppen climate classification was not only...
helpful to determine the influence of specific climatic factors (amount and distribution of precipitation, temperature) on components, but also solved the problem of planting area differences. These data provided a theoretical basis for the directional cultivation of walnuts. Based on correspondence analysis, we confirmed component differences among cultivars and further distinguished cultivars from components. Moreover, combined with k-means clustering and specific nutritive characters, the analyzed cultivars achieved an unambiguous classification (high protein, high antioxidants, high-quality oil, and middle components) in terms of commercial values and food industry applications. The health-promoting properties of phytochemical components in walnuts were fully determined.

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CONFLICT OF INTEREST
The author declares that there are no conflicts of interest.

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