

Research Article

Determining the Geographical Origin of Fuji Apple from China by Multivariate Analysis Based on Soluble Sugars, Organic Acids, and Stable Isotopes

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The aim of this study was to explore the regional characteristics of soluble sugars, organic acids, and stable isotopes ($\delta^2\text{H}$, $\delta^{18}\text{O}$, and $\delta^{13}\text{C}$) in Fuji apple and the viability of tracing the geographical origin. Totally, 181 Fuji apple samples from 2017 and 2018 from three main apple production regions in China, Bohai Bay (BHB), Loess Plateau (LP), and Northwest region (NW) were collected. The parameters of soluble sugars, organic acids, and stable isotopes in samples were analyzed with HPLC, IC, and IRMS, respectively. The results of regional difference analysis, multiway variance analysis, and correlation analysis indicated that sorbitol (Sor), glucose (Glu), fructose (Fru), sucrose (Sucr), $\delta^2\text{H}$, and $\delta^{13}\text{C}$ can be used to distinguish the samples from the three regions. Stepwise linear discriminant analysis (SLDA) showed that the correct discriminant rate of samples from the advantageous production areas of apples in China (BHB and LP) was 82.2%, and the most effective indexes were Glu, Fru, Sucr, and $\delta^2\text{H}$. Moreover, satisfactory classification can be achieved in samples from BHB and NW, with a correct classification rate of 90.0%, and Sor, Glu, and Fru were included in the discrimination model. Furthermore, the validity of the discriminant model was verified by the prediction set. The study also found that organic acids were not suitable to distinguish the apple samples from the three regions. In addition, soluble sugars and stable isotopes could not effectively distinguish LP and NW samples, which was also the reason that the samples from the three main apple production regions could not be distinguished well.

1. Introduction

In recent years, due to the occurrence of food safety issues such as heavy metal pollution, pesticide residues, adulteration, and fake and inferior agricultural products [1], consumers want to know more relevant information to confirm the safety of the food they purchase [2]. In this situation, consumers are more inclined to buy agricultural products

with a clear geographical origin, especially certified foods, such as agricultural products with geographical indications, which comply with relevant standards [3, 4]. However, some unauthorized operators may use false labels to impersonate agricultural products with clear geographical indications in order to obtain higher profits, as the agricultural products with guaranteed origin will charge a high premium, which will damage the interests of operators and consumers [5].

Therefore, the development of suitable analytical techniques for tracing the origin of agricultural products is highly desirable for consumers, production operators, and government regulators [6].

Due to the different principles and characteristics, especially the limitations of technology in the application, it is difficult to accurately trace the origin of agricultural products with only one traceability technology [7–9]. For example, stable isotope ratios analysis technology is an increasingly accepted tool for tracing the geographical origin of agricultural products, but the isotopic composition is mainly affected by geographic and climatic conditions, such as latitude, distance from the sea, altitude, temperature, and precipitation [10]. Therefore, the isotopic compositions of agricultural products from areas with similar climates and topography may not be effectively distinguished [5]. Using organic fingerprints to trace the origin of agricultural products, the discriminant rates were varied greatly due to the selected indexes. Therefore, screening out the organic ingredients which are unique or closely related to environmental conditions is the key to use organic compounds fingerprint analysis technology to trace the origin of agricultural products [11]. Therefore, it is the current development trend to use multiple technologies and multiple indexes to trace the origin of agricultural products. For instance, by combining the stable isotopes with volatile compounds, organic compounds, and multiple elements, the geographical origin of potatoes, kiwifruit, apple juices, and red wine can be well classified, and the total correct discrimination rate was varied from 83.9% to 100% [8, 12–14].

The cultivation of apples in China is mainly distributed in the advantageous production areas of apple, Bohai Bay (BHB), and Loess Plateau (LP), including the seven major apple-producing provinces of Shaanxi, Shandong, Henan, Shanxi, Hebei, Liaoning, and Gansu. In 2018, the apple cultivated area and the yield in BHB and LP accounted for 84.22% and 89.12% of the total cultivated area and yield in China [15]. Xinjiang and Ningxia are characteristic apple-producing areas in China and belong to the Northwest region (NW). In recent years, the cultivation area and yield of apples in the NW have continued to increase; moreover, with the changes in the layout of apple production, Xinjiang has gradually become an important apple-producing area in China. Furthermore, the Fuji apple is the main cultivated variety, and its cultivation proportion accounts for 72.7% of the total cultivated area [16]. As we all know, the quality and price of agricultural products will be different due to their geographic origin [17]; in addition, studies have found that the physiological quality of apples was related to the latitude, longitude, and altitude of the growing area [18]. For example, fructose has the highest proportion in the soluble sugars in Fuji apple from Shaanxi Province which belongs to the LP [19], with a higher price than in other provinces; moreover, apples in some specific areas of Shaanxi have been recognized as “protected designation of origin” (PDO) products [20]. Therefore, tracing the origin of the Fuji apple in the three main apple production regions in China is of great significance to achieving the traceability of apple and protecting the regional advantages of agricultural products.

What’s more, there has not been literature that specifically studied the use of organic compounds and stable isotope fingerprint characteristics to trace the origin of the Fuji apple.

Since the isotope ratios of carbon, hydrogen, and oxygen were altitude-, latitude-, and climate-dependent, these three stable isotope ratios can provide information about the geographic origin and metabolism of plants, so the isotopic ratios of carbon, hydrogen, and oxygen are suitable for tracking the geographical origin of agricultural products related to regional climate conditions [17, 21–23]. In addition, soluble sugars and organic acids were important nutrients in apples, as well as the main flavor compounds in apples. Moreover, studies have found that soluble sugars and organic acid compounds in Fuji apple were different between regions [19]. Therefore, the soluble sugars, organic acids, and stable isotopes ($\delta^2\text{H}$, $\delta^{18}\text{O}$, and $\delta^{13}\text{C}$) will be used to study the effectiveness of multi-index in tracking the origin of Fuji apples from the three main apple producing areas (BHB, LP, and NW) in China. It is expected to provide references for tracing the origin of apples.

2. Materials and Methods

2.1. Sample Collection. A total of 181 Fuji apple samples were collected from the 2017 and 2018 harvests from three main apple production regions in China, BHB (Hebei, Shandong, and Liaoning provinces), LP (Shaanxi, Gansu, Shanxi, and Henan provinces), and NW (Ningxia and Xinjiang autonomous regions). For each sample, approximately 5 kg of fruit was collected at the harvest maturity stage from September to November. Samples were transported to the laboratory 3 days after collection. The information on samples was shown in Table 1.

2.2. Sample Preparation. About 2.0 kg of fruit was ground into a powder in liquid nitrogen by a SPEX Sample Prep system (New Jersey, USA) and stored at -20°C for the determination of soluble sugars and organic acids. For the rest of the samples, wipe the surface of the peel and core; then the sample was homogenized by a homogenizer (JT C, Jintian, China), which was used for the determination of stable isotope ratios. The extraction of soluble sugars and organic acids was carried out according to the previously reported method [24, 25] and the extracted solution was stored at -20°C and analyzed by the instrument later. The homogenized sample was placed in the sample chamber of a vacuum freeze dryer (VFD-1000, Bilon, China); then, the sample was freeze-dried for 72 hours under the instrument conditions of -40°C and under 15pa. The dried sample was passed through a sieve (100 mesh, Huafeng, China) immediately. Finally, a powdered sample was obtained to detect the isotope ratio $^{13}\text{C}/^{12}\text{C}$ in samples.

2.3. Instrument Conditions

2.3.1. High-Performance Liquid Chromatography (HPLC) Measurement Conditions [25,26]. After filtered through a

TABLE 1: The region information of apple samples.

Location	Province/region	Longitude (E)	Latitude (N)	Altitude (m)	Years	Mean annual temperature (°C)	Annual precipitation (mm)	Number of samples
BHB	Hebei	113°27'–119°50'	36°05'–42°40'	3–386	2017	11.3	499.6	14
					2018	11.8	549.0	15
BHB	Liaoning	118°53'–125°46'	38°43'–43°26'	10–39	2017	10.3	368.0	2
					2018	10.6	477.5	3
BHB	Shandong	114°19'–122°43'	34°22'–38°23'	33–305	2017	13.5	622.4	2
					2018	14.2	750.1	9
LP	Shaanxi	105°29'–111°15'	31°42'–39°35'	580–969	2017	19.0	591.0	23
					2018	16.9	525.6	23
LP	Gansu	92°13'–108°46'	32°31'–42°57'	1085–1421	2017	9.8	546.1	1
					2018	13.5	725.8	5
LP	Shanxi	110°14'–114°33'	34°34'–40°43'	365–778	2017	13.8	503.6	9
					2018	17.7	386.0	7
LP	Henan	110°21'–116°39'	31°23'–36°22'	504–626	2017	15.0	641.1	3
					2018	14.8	525.7	4
NW	Ningxia	104°17'–107°39'	35°14'–39°23'	1111–1226	2017	10.8	224.5	24
					2018	10.6	280.1	8
NW	Xinjiang	73°40'–96°23'	34°22'–49°10'	663–1104	2017	12.2	70.2	17
					2018	11.6	101.4	12

* BHB, the Bohai Bay; LP, the Loess Plateau; NW, Northwest Region.

0.22 μm pore filter (Fine, Japan), the extracted solution was used to detect the organic acid compounds directly, using HPLC (LC-10A, Shimadzu, Japan), with SPD-10A UV-VIS detector, and a C18 column (Ultimate LP-C18, 4.6 mm \times 300 mm, 5 μm , Ultimate, China). A total of five organic acid compounds, including oxalic acid (Oxa), tartaric acid (Tar), quinic acid (Qui), malic acid (Mal), and shikimic acid (Shi), were identified by comparison with the standards. The calibration curves were prepared by plotting different concentrations ranging from 0.05 to 50 mg/L (for Mal, the concentrations ranging from 25 to 400 mg/L) of standards versus the area measurements in UPLC ($R^2 \geq 0.9992$). Results were expressed as mg/g. The reproducibility of the chromatographic separation of the organic acid compounds was carried out according to the previously reported method (Wu et al., Wang). The results of the reproducibility of chromatographic separation for organic acids were expressed as relative standard deviation (RSD%) as follows: 0.63 for Oxa, 1.41 for Tar, 0.43 for Qui, 0.62 for Mal, and 0.81 for Shi. The content of total acid in the samples was the sum of the contents of the five organic acids.

2.3.2. Ion Chromatograph (IC) Measurement Conditions [24, 25]. The soluble sugars were detected by an Ion Chromatograph (ICS-5000, Dionex, USA) with a conductivity detector, an anion exchange analytical column (Dionex CarboPac™ PA10, 4 mm \times 250 mm, Thermo Fisher Scientific, USA), and a guard column (IonPac AG23, 4 mm \times 50 mm, Dionex, USA). The extracted solution after being diluted 50 times with deionized water can be used to measure sorbitol (Sor), glucose (Glu), fructose (Fru), and sucrose (Sucr) in the samples. The soluble sugars were identified by comparison with the standards. The calibration curves were prepared by plotting different concentrations ranging from 0.5 to 100 mg/L of standards versus the area measurements in IC ($R^2 \geq 0.9965$). The result was expressed

as mg/g. The results of the reproducibility of chromatographic separation for soluble sugars, expressed as RSD%, were as follows: 1.75 for Sucr, 1.52 for Glu, 1.69 for Fru, and 0.37 for Sor. The content of total sugar in the samples was the sum of the contents of the four soluble sugars.

2.3.3. Isotopic Ratio Mass Spectrometer (IRMS) Measurement Conditions

- (1) **Stable Hydrogen and Oxygen Isotope Determination Conditions** [27,28]. About 2 g of the homogenized sample was weighed and placed in a glass sample bottle. The fully automatic vacuum condensed water extraction system (LI-2100, LICA, China) was used to extract the water in the samples; then, the extracted water was put into the automatic sampling tray of the element analyzer (Flash HT2000, Thermo Fisher Scientific, USA). The elements of H and O in the samples were converted into H_2 and CO by splitting at 1,400°C. After passing through the chromatographic column, H_2 and CO successively entered the stable isotope mass spectrometer (MAT 253, Thermo Fisher Scientific, USA) to detect the isotope ratios $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$. The flow rates of helium carrier gas and helium purge gas were both 100 mL/min.
- (2) **Stable Carbon Isotope Determination Conditions** [27,28]. About 0.8 mg of the powder sample was weighed, which was wrapped in a tin cup, and placed in the element analyzer. The element of C in the sample was converted into CO_2 in a combustion furnace at 960°C. After passing through the chromatographic column, CO_2 entered the stable isotope mass spectrometer for the determination of the isotope ratio $^{13}\text{C}/^{12}\text{C}$. The flow rates of helium carrier gas and helium purge gas were both 100 mL/min.

(3) *The Calculated Stable Isotope Ratios.* The isotope values were calibrated against standard materials, which were Vienna Standard Mean Ocean Water (V-SMOW), USGS45 ($\delta^2\text{HVSOW} = -10.3\text{‰}$, $\delta^{18}\text{OVSMOW} = -2.238\text{‰}$), USGS46 ($\delta^2\text{HVSOW} = -235.8\text{‰}$, $\delta^{18}\text{OVSMOW} = -29.8\text{‰}$), and USGS40 ($\delta^{13}\text{CVPDB-LSVEC} = -26.39\text{‰}$, $\delta^{14}\text{NAIR} = -4.52\text{‰}$). According to the equation, R was the isotope ratio of heavy isotope to light isotope abundance in the samples ($^{18}\text{O}/^{16}\text{O}$, $^2\text{H}/^1\text{H}$, and $^{13}\text{C}/^{12}\text{C}$). R_{sample} and R_{standard} were the isotope ratio of the samples and the international standards. Results were referenced to Vienna Pee Dee Belemnite (V-PDB) for $\delta^{13}\text{C}$ and Vienna Standard Mean Ocean Water (V-SMOW) for $\delta^2\text{H}$ and $\delta^{18}\text{O}$. The analytical precision was 2‰, 0.4‰, and 0.2‰ for H, O, and C, respectively. The stable isotope ratios were calculated as follows:

$$\delta(\text{‰}) = \left[\frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \right] \times 1000 \quad (1)$$

2.4. Statistical Analysis. When data conformed to both normality and homogeneity assumptions, a regional different analysis was performed by one-way analysis of variance (ANOVA) (P value < 0.05), and the Pearson correlation analysis was used to investigate the relationship between indexes and regional conditions. When data conformed to the normality assumption but failed on the homogeneity of variances, analysis of variance was performed using Welch's test. For not normally distributed data, Kruskal-Wallis (K-W) was used for the analysis of regional differences, and Spearman correlation analysis was used to investigate the relationship between indexes and regional conditions. Multiway ANOVA was applied to quantify the contributions of geographical origin, harvest year, and their interactions to the total variance in stable isotope ratio, soluble sugars, and organic acids levels. In addition, the sample set was randomly split up into two groups, with three-quarters of the total samples as a training set and the remaining one-quarter of samples as a prediction set. According to F-value, the training set was used to create the discriminant model by Linear Discriminant Analysis (LDA), and the predictive ability of the discriminant model was estimated by the prediction set. Stepwise linear discriminant analysis (SLDA) was used to select necessary and optimal indexes to incorporate into the discriminant model, and the coefficients of the indexes in the discriminant model were standardized coefficients. The statistical analysis of the data was performed with SPSS 26.0 software (SPSS, IBM Corp., USA).

3. Results

3.1. Analysis of Soluble Sugars in Apples. The proportion of each sugar component in samples from high to low was Fru, Sucr, Glu, and Sor. For BHB, LP, and NW samples, the proportion of Fru to total sugars was 45.93%, 49.65%, and

49.33%, respectively (Figure 1(a)), which was the main soluble sugar component, in addition, the proportion of Sucr (26.44%, 23.03%, and 28.36%, respectively) was higher than Glu (23.12%, 22.51%, and 16.19%, respectively), and the lowest content was Sor (4.51%, 4.81%, and 6.12%, respectively). Moreover, the radar chart showed that the soluble sugar content in samples from different regions had different patterns (Figure 2(a)).

Regional differences of soluble sugars were shown in Table 2, and the contents of soluble sugars were expressed as means \pm standard deviation (SD). In LP samples, the average content of Fru was 59.5 ± 12.8 mg/g FW, which was higher than in BHB and NW samples, which are 54.3 ± 9.41 mg/g FW and 55.2 ± 13.3 mg/g FW, respectively. The average content of Sucr in LP samples was 27.4 ± 9.21 mg/g FW, which was lower than BHB and NW samples (31.6 ± 9.34 mg/g FW and 32.2 ± 10.0 mg/g FW, respectively). The contents of Fru and Sucr in the LP samples were significantly different from the BHB and NW samples ($P < 0.05$), and the content of Sucr was significantly different in LP and NW samples ($P < 0.01$), while the differences in Fru and Sucr contents in BHB and NW samples were not significantly ($P > 0.05$). The content of Glu in NW samples was significantly different from that in BHB and LP samples ($P < 0.01$). In addition, the content of Sor was significantly different between the BHB and NW samples ($P < 0.05$).

3.2. Analysis of Organic Acids in Apples. Different from soluble sugars, the content characteristics of organic acids had similar patterns among regions, and the content of organic acids in descending order was Mal, Qui, Oxa, Tar, and Shi (Figure 2(b)). In the samples from BHB, LP, and NW, Mal accounted for 85.18%, 85.66%, and 88.73% of the total acids, respectively (Figure 1(b)), indicating that Mal occupies an absolute advantage in the total acid content.

Regional differences of organic acids are shown in Table 2, and the contents of organic acids were expressed as means \pm standard deviation (SD). Mal was the main organic acid component in Fuji apple; the average content of Mal in BHB, LP, and NW samples was 4.11 ± 1.31 mg/g FW, 3.70 ± 0.828 mg/g FW, and 3.85 ± 1.20 mg/g FW, respectively; however, the differences in the three regions were not significant ($P > 0.05$). The average content of Qui in the BHB samples was significantly different from NW samples ($P < 0.01$). The content of Tar in NW samples was significantly different from that in BHB and LP samples ($P < 0.05$). The average content of Oxa and Shi in samples from the three main production regions was not significantly different ($P > 0.05$).

3.3. Analysis of Stable Isotopes in Apples. The characteristics of the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values in the samples from the three main apple-producing areas showed similar patterns, while the $\delta^2\text{H}$ value in the BHB samples was higher than that in NW and LP samples (Figure 2(c)). The mean $\delta^2\text{H}$ value of the BHB samples was -39.6‰ , which was higher than that of the LP and NW samples (-44.6‰ and -44.4‰ , respectively), and the results of ANOVA showed that the $\delta^2\text{H}$ value

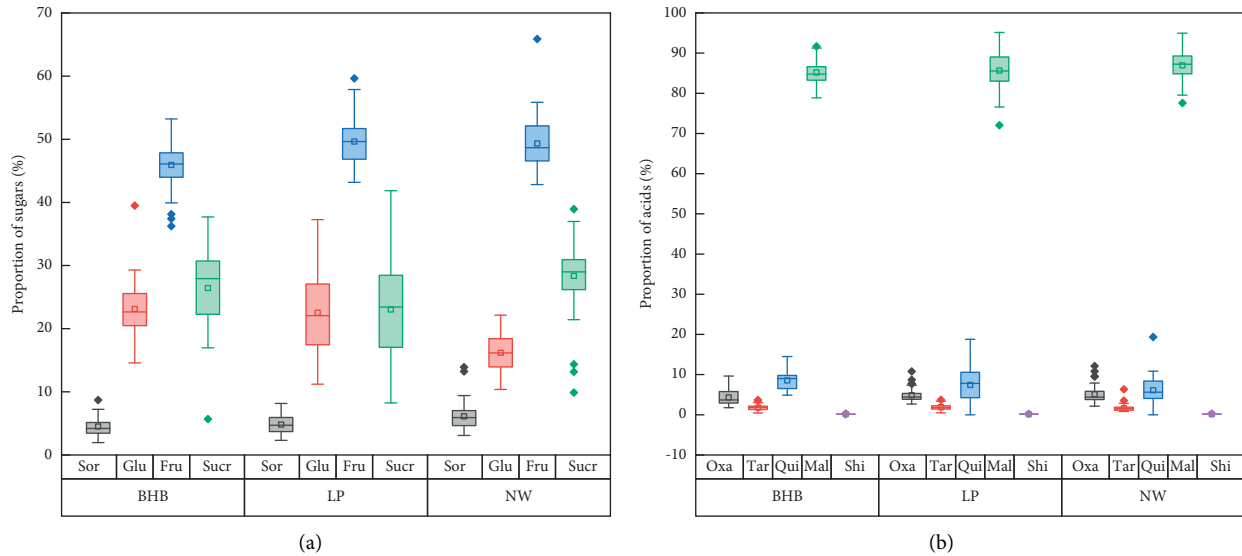


FIGURE 1: The proportions of soluble sugars (a) and organic acids (b) in samples.

of BHB samples was different from LP and NW samples, and the difference was significant ($P < 0.01$). In addition, there was no significant difference in the mean $\delta^2\text{H}$ values between LP and the NW samples ($P > 0.05$). The distribution of $\delta^{18}\text{O}$ values in the samples was similar to the $\delta^2\text{H}$ value; the mean $\delta^{18}\text{O}$ value of BHB samples was higher than that of LP and NW samples, which were -4.67% , -5.26% , and -5.22% , respectively. The results of ANOVA showed that the mean $\delta^{18}\text{O}$ value in the BHB samples was significantly different from the LP sample ($P < 0.05$). In addition, the mean $\delta^{18}\text{O}$ value of NW samples was not significantly different from BHB and LP samples ($P > 0.05$). The distribution of the $\delta^{13}\text{C}$ value of samples roughly showed that the $\delta^{13}\text{C}$ value of the NW samples was higher than that of LP samples, while the $\delta^{13}\text{C}$ value of the BHB samples was the lowest, and the mean $\delta^{13}\text{C}$ values of NW, LP, and BHB samples were -25.5% , -25.7% , and -26.3% , respectively. The results of ANOVA analysis showed that the mean $\delta^{13}\text{C}$ value of BHB samples was different from the LP and NW samples, and the difference was significant ($P < 0.01$). In addition, the mean $\delta^{13}\text{C}$ value of LP and NW samples was not significantly different ($P > 0.05$) (Table 2).

3.4. Multiway ANOVA. A combined analysis of variance of two years and three geographical origins was performed using the general linear model (GLM). Harvest year and geographical origin were considered as the fixed factors. The effects were partitioned into different sources: year, region, and region \times year (Table 3). Oxa was not statistically significant in this analytical model ($P > 0.05$). Sor, Glu, Fru, Sucr, Tar, Qui, Mal, Shi, $\delta^2\text{H}$ value, and $\delta^{18}\text{O}$ value were significantly influenced by year ($P < 0.01$). Sor, Glu, Fru, Sucr, $\delta^2\text{H}$ value, and $\delta^{13}\text{C}$ value were significantly affected by region ($P < 0.01$). Qui, Mal, and $\delta^{18}\text{O}$ values were significantly affected by region ($P < 0.05$). Region \times year had a significant effect on Sucr ($P < 0.05$) and $\delta^{13}\text{C}$ ($P < 0.01$). The results showed that geographical origin was the most

important source of the concentration variations of $\delta^{13}\text{C}$ value, and the contribution percentage was 44.76%. The harvest year was the most source of variation for soluble sugars, organic acids, $\delta^2\text{H}$, and $\delta^{18}\text{O}$.

3.5. Correlation between Indexes and Regional Conditions. To identify the relationship between indexes and regional conditions (longitude, latitude, altitude, mean annual temperature, and annual precipitation), correlation analysis was employed to assess whether or not positive/negative correlation patterns existed. The results showed that longitude was significantly negatively correlated with Sor ($r = -0.150$, $P < 0.05$) and $\delta^{13}\text{C}$ value ($r = -0.244$, $P < 0.01$) had a significant positive correlation with Glu ($r = 0.320$, $P < 0.01$) and $\delta^2\text{H}$ value ($r = 0.228$, $P < 0.01$) (Table 4). Latitude was negatively correlated with Glu ($r = -0.328$, $P < 0.01$) and Fru ($r = -0.205$, $P < 0.01$) and significantly positively correlated with Sucr ($r = 0.189$, $P < 0.05$). Altitude was significantly positively correlated with Sor ($r = 0.290$, $P < 0.01$) and $\delta^{13}\text{C}$ value ($r = 0.336$, $P < 0.01$) and was correlated with Glu ($r = -0.326$, $P < 0.01$) and Tar ($r = -0.170$, $P < 0.05$) showing a significant negative correlation. The temperature was significantly positively correlated with Glu ($r = 0.199$, $P < 0.01$) and was significantly negatively correlated with Sucr ($r = -0.222$, $P < 0.01$) and Mal ($r = -0.162$, $P < 0.05$). There was a significant negative correlation between precipitation and Sor ($r = -0.196$, $P < 0.01$) and $\delta^{13}\text{C}$ value ($r = -0.178$, $P < 0.05$), and there was a significant positive correlation with Glu ($r = 0.398$, $P < 0.01$), Tar ($r = 0.160$, $P < 0.05$), and $\delta^2\text{H}$ value ($r = 0.181$, $P < 0.05$). In addition, in the sample collection area, Oxa, Qui, Shi, and $\delta^{18}\text{O}$ values had no correlation with the regional conditions studied. Therefore, geographical location has a significant impact on most soluble sugars, organic acids, and stable isotopes studied ($P < 0.05$), which provides a basis for distinguishing the samples from different geographical regions by using soluble sugars, organic acids, and stable isotopes.

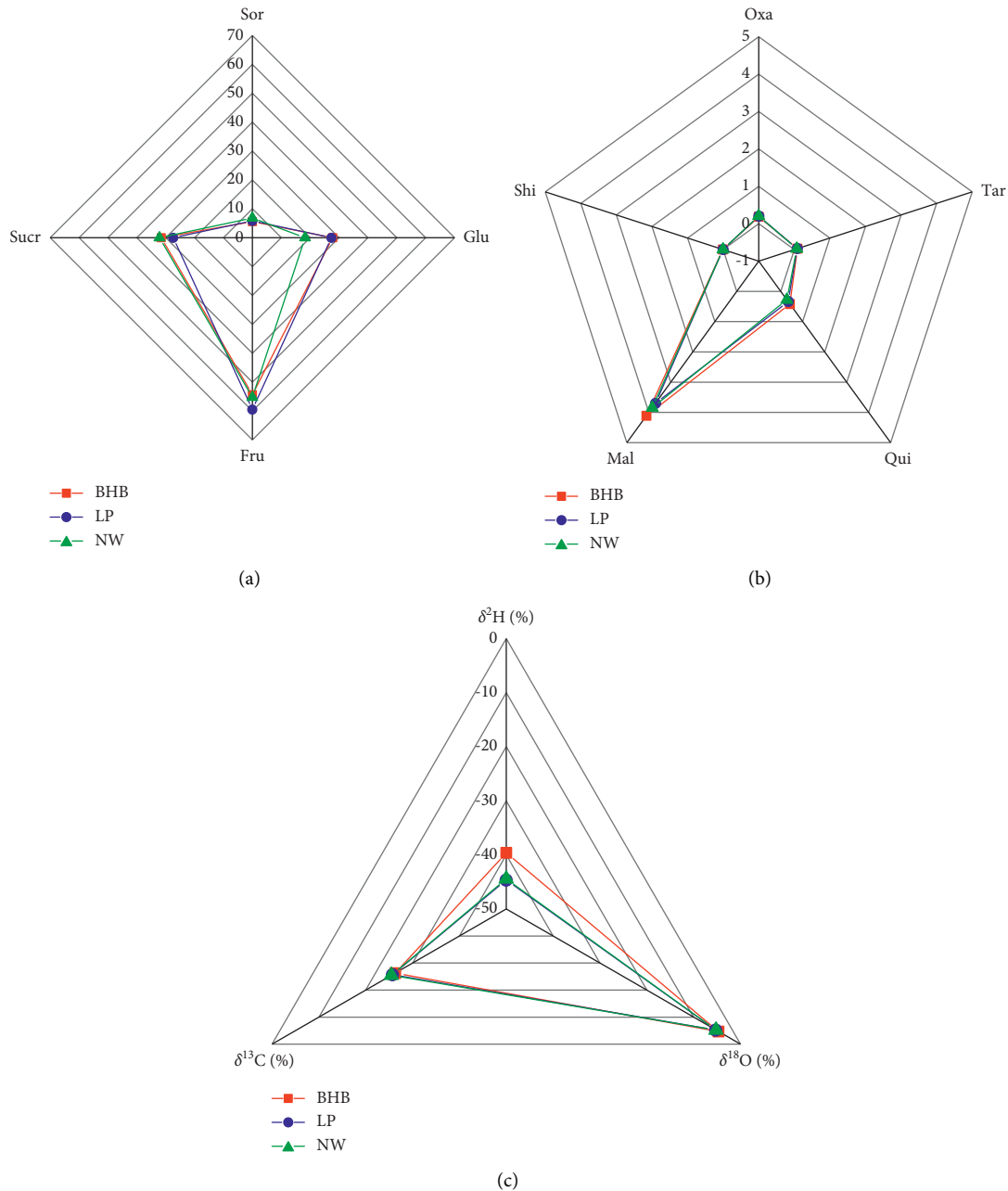


FIGURE 2: The content characteristics of soluble sugars (a), organic acids (b), and stable isotopes (c) in samples from different regions.

3.6. Linear Discriminant Analysis (LDA). In order to explore the effectiveness of soluble sugars, organic acids, and stable isotopes in tracing the origin of the Fuji apple in China, the indexes had significant differences between regions ($P < 0.05$) and were significantly affected by regional factors ($P < 0.05$); besides, the selected indexes were significantly correlated with the regional conditions. Finally, Sor, Glu, Fru, Sucr, $\delta^2\text{H}$, and $\delta^{13}\text{C}$ were selected for stepwise discriminant analysis (SLDA) of BHB, LP, and NW samples. About three-quarters of the samples (136 samples) were used as training set and the remaining 45 samples, BHB (11 samples), LP (19 samples), and NW (15 samples), were used as prediction set to verify the stability of the discriminant models.

SLDA was used to classify Fuji apple samples from BHB, LP, and NW. The classification of the training set by the canonical discriminant functions was shown in Figure 3; it can be seen that BHB, NW, and LP samples were overlapped, mainly because the LP samples were misjudged as BHB and NW samples, and the correct classification rate and cross-validation rate were only 69.1% and 67.6% (Table 5); in addition, Sor, Glu, Fru, Sucr, and $\delta^2\text{H}$, which had a significant impact on sample discrimination ($P < 0.001$), were included in the discriminant model, and the correct discrimination rate of the discriminant model for the prediction set was only 75.6% (for BHB, NW, and LP samples was 81.8%, 80.0%, and 68.4%, respectively) (Table 6). In addition,

TABLE 2: Analysis of the regional differences of soluble sugars, organic acids, and stable isotopes in samples.

Composition	Location	Means \pm SD	Range	Variation coefficient (%)
Sor	BHB	5.57 \pm 2.76 b	2.04–14.4	49.5
	LP	5.86 \pm 2.22ab	1.77–10.9	37.8
	NW	6.81 \pm 2.75a	2.39–14.4	40.4
Glu	BHB	27.8 \pm 8.03a	12.8–48.9	28.9
	LP	27.3 \pm 10.2a	11.4–52.0	37.4
	NW	18.2 \pm 5.82 b	8.90–34.8	31.9
Fru	BHB	54.3 \pm 9.41 b	38.2–87.0	17.3
	LP	59.5 \pm 12.8a	34.0–106.1	21.6
	NW	55.2 \pm 13.3 b	36.6–126	24.0
Sucr	BHB	31.6 \pm 9.34a	5.21–53.2	29.6
	LP	27.4 \pm 9.21 b	9.59–49.1	33.6
	NW	32.2 \pm 10.0a	6.74–74.5	31.1
Oxa	BHB	0.187 \pm 0.0417a	0.110–0.301	22.3
	LP	0.204 \pm 0.0686a	0.0811–0.641	33.6
	NW	0.207 \pm 0.0839a	0.108–0.482	40.6
Tar	BHB	0.0852 \pm 0.0368a	0.0191–0.175	43.2
	LP	0.0812 \pm 0.0318a	0.0191–0.143	39.1
	NW	0.0684 \pm 0.0331 b	0.0295–0.180	48.4
Qui	BHB	0.416 \pm 0.171a	0.146–0.901	41.1
	LP	0.339 \pm 0.216ab	0.000–0.943	63.7
	NW	0.276 \pm 0.230 b	0.00–1.58	83.4
Mal	BHB	4.11 \pm 1.31a	2.00–7.72	31.8
	LP	3.70 \pm 0.828a	1.79–6.10	22.4
	NW	3.85 \pm 1.20a	2.12–8.60	31.3
Shi	BHB	0.00670 \pm 0.00137a	0.00460–0.0110	20.4
	LP	0.00631 \pm 0.00146a	0.00310–0.0103	23.2
	NW	0.00632 \pm 0.00169a	0.00360–0.0105	26.8
$\delta^2\text{H}$	BHB	–39.6 \pm 6.88a	–57.6––21.8	17.4
	LP	–44.6 \pm 5.77b	–59.5––32.7	12.9
	NW	–44.4 \pm 7.66b	–73.9––25.6	17.2
$\delta^{18}\text{O}$	BHB	–4.67 \pm 1.44a	–8.48––1.85	30.9
	LP	–5.26 \pm 1.57b	–8.58––1.59	29.8
	NW	–5.22 \pm 1.51ab	–8.26––1.86	28.9
$\delta^{13}\text{C}$	BHB	–26.3 \pm 0.973b	–28.1––24.5	3.70
	LP	–25.7 \pm 0.834a	–28.0––23.3	3.25
	NW	–25.5 \pm 0.963a	–27.3––22.3	3.78

* The different letters in the same column mean significant difference ($P < 0.05$); the coefficient of variation of stable isotopes was the absolute value of the original value.

it was found that the classification effect of the samples from two producing areas was better (Figure 3). So, SLDA was performed on samples from the two production regions to explore the effectiveness of the combination of soluble sugars and stable isotopes in distinguishing the regions of samples.

Glu, Fru, Sucr, and $\delta^2\text{H}$ were selected by SLDA to classify Fuji apple samples from the advantageous production areas of apple in China (BHB and LP). The correct classification rate and cross-validation rate of the training set of BHB and LP samples were both 82.2% (Table 5), and the correct discrimination rate of the prediction set was 80.0% (Table 6). When SLDA was used to classify Fuji apple samples from BHB and NW, a satisfactory classification was obtained, and the correct classification rate of the training set and cross-validation rate were 90.0% and 86.3%, respectively (Table 5); moreover, the discriminant model, including Sor, Glu, and Fru, had a correct discrimination rate of 84.6% for prediction set (Table 6). However, it was found that the samples from the LP and NW were not easy to be distinguished, and

the correct classification rate of the training set was only 71.6% (Table 5), which was the reason that the classification of samples from the three production regions was not satisfactory.

4. Discussion

In this study, Fru was the main soluble sugar in samples, accounting for 45.93%–49.65% of the total sugar content, and Mal was the main organic acid, accounting for 85.18%–88.73% of the total acid, which was consistent with previous studies [24,29]. For apple fruits, the formation and accumulation of the flavor substances (soluble sugars and organic acids) were closely related to ecological and climatic conditions such as temperature, light intensity, and altitude. In this study, LP (Shaanxi, Gansu, Shanxi, and Henan) is next to BHB (Shandong, Hebei, and Liaoning) and NW (Ningxia and Xinjiang). The altitude and annual average temperature of provinces on the LP are similar to those of BHB and NW, such as Gansu, Ningxia, and Xinjiang. This may cause the

TABLE 3: The contribution of each factor to the content variability of soluble sugars, organic acids, and stable isotopes.

Dependent variable	Calibrate model			Year			Region			Region × year			Error Contribution rates (%)
	F-Value	Significance (p value)	Contribution rates (%)	Significance (p value)	Contribution rates (%)	Significance (p value)	Contribution rates (%)	Significance (p value)	Contribution rates (%)	Significance (p value)	Contribution rates (%)		
Sor	5.469	≤0.001	70.22	≤0.001	24.93	0.002	1.10	0.746	3.75				
Glu	47.318	≤0.001	82.29	≤0.001	15.70	≤0.001	1.37	0.125	0.65				
Fru	23.314	≤0.001	92.69	≤0.001	5.37	0.005	0.94	0.389	0.99				
Sucr	4.527	≤0.001	43.37	0.005	35.09	0.002	16.25	0.049	5.29				
Oxa	1.137	0.343	34.91	0.186	0.07	0.996	45.25	0.105	19.77				
Tar	46.865	≤0.001	98.09	≤0.001	0.50	0.337	0.95	0.132	0.46				
Qui	57.677	≤0.001	96.90	≤0.001	1.60	0.021	1.09	0.071	0.40				
Mal	22.227	≤0.001	93.43	≤0.001	3.33	0.026	2.35	0.074	0.89				
Shi	21.697	≤0.001	95.85	≤0.001	1.96	0.130	1.24	0.273	0.95				
δ ² H	7.315	≤0.001	53.56	≤0.001	35.32	≤0.001	6.64	0.229	4.47				
δ ¹⁸ O	39.428	≤0.001	96.64	≤0.001	2.17	0.025	0.62	0.345	0.58				
δ ¹³ C	8.473	≤0.001	15.12	0.077	44.76	≤0.001	35.35	≤0.001	4.77				

TABLE 4: Correlations of soluble sugars, organic acids, and stable isotopes with regional conditions.

	Sor	Glu	Fru	Sucr	Oxa	Tar	Qui	Mal	Shi	$\delta^2\text{H}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$
Longitude	-0.150*	0.320**	0.023	-0.002	-0.045	-0.012	0.121	0.090	-0.045	0.228**	0.081	-0.244**
Latitude	0.017	-0.328**	-0.205**	0.189*	-0.100	-0.039	-0.005	0.006	0.132	-0.112	-0.032	-0.035
Altitude	0.290**	-0.326**	0.140	0.073	0.114	-0.170*	-0.090	0.020	-0.073	-0.123	-0.011	0.336**
Mean annual temperature	-0.065	0.199**	0.051	-0.222**	0.022	0.070	-0.070	-0.162*	-0.127	0.045	-0.049	0.056
Annual precipitation	-0.196**	0.398**	0.069	-0.129	-0.063	0.160*	0.112	0.080	0.058	0.181*	0.076	-0.178*

**Significant correlation ($P < 0.01$). *Significant correlation ($P < 0.05$).

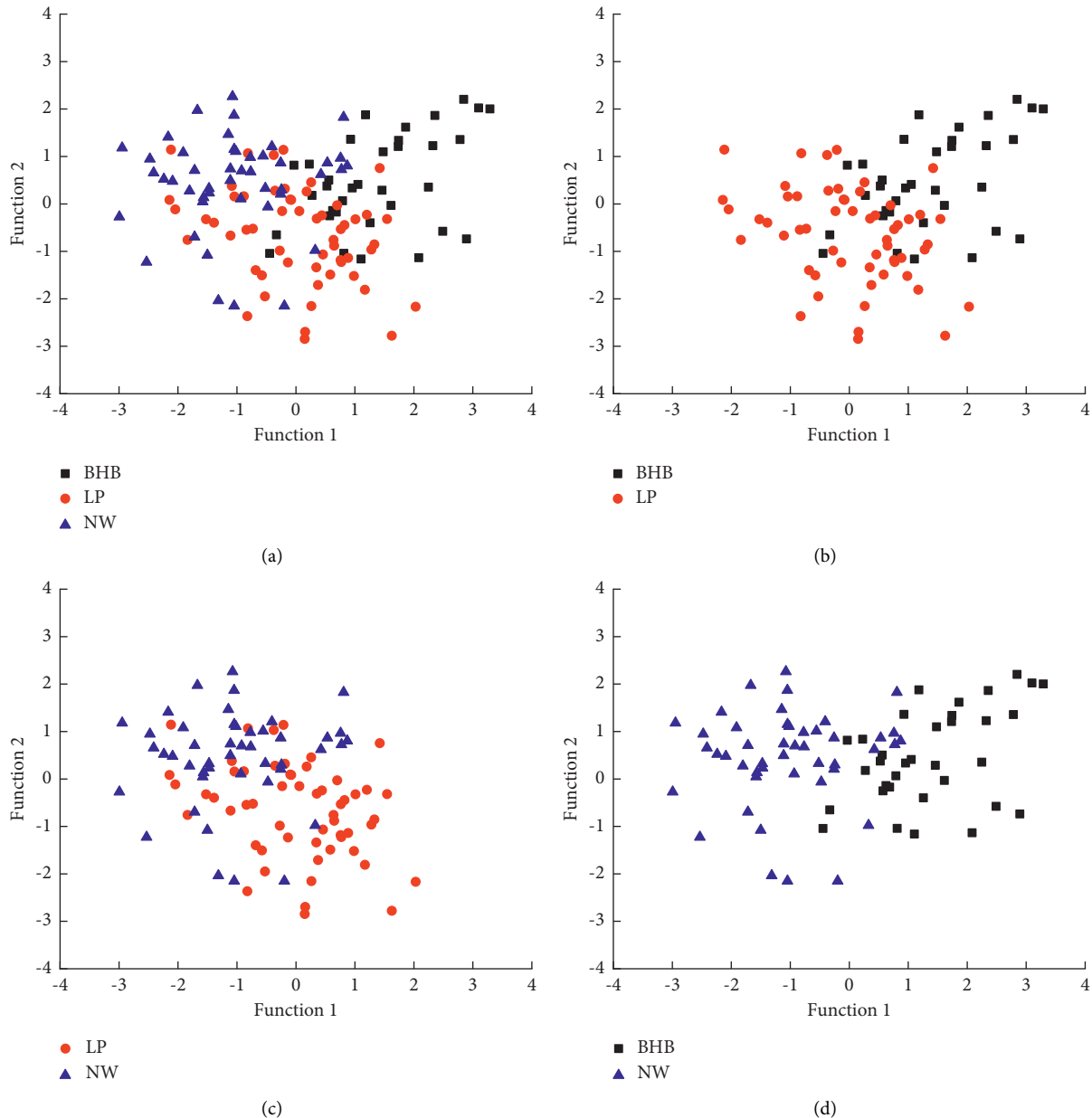


FIGURE 3: Scatter plot of the scores of apple samples from the three major apple-producing areas on the canonical discriminant functions.

content of soluble sugar in the LP sample to be similar to the NW and BHB samples, making it difficult to be classified from BHB and NW samples. In fact, acid metabolism and regulation in apples are very complex, and the acid content is

jointly regulated by the relevant genes and enzymes [30]. In addition, it is also affected by many factors such as environment and cultivation conditions. For example, a certain degree of deficit irrigation will affect the content of total acid

TABLE 5: The correct classification rate (%) of the training set obtained by SLDA.

Sample groups	BHB	LP	NW	Total	
				Original	Cross-validated
BHB-LP-NW	73.5	60.7	76.1	69.1	67.6
BHB-LP	73.5	87.5		82.2	82.2
LP-NW		71.4	71.7	71.6	71.6
BHB-NW	91.2		89.1	90.0	86.3

TABLE 6: The correct discrimination rate of discrimination model for prediction set.

Sample groups	Discriminant models	Prediction set (%)	
		Total	
BHB-LP-NW	BHB = $-0.653 \text{ Sor} + 1.973 \text{ Glu} - 1.829 \text{ Fru} + 1.468 \text{ Sucr} + 0.859 \delta^2\text{H} - 2.166$	81.8	75.6
	LP = $-0.134 \text{ Sor} + 0.285 \text{ Glu} + 0.448 \text{ Fru} - 0.568 \text{ Sucr} - 0.477 \delta^2\text{H} - 1.313$	68.4	
	NW = $0.646 \text{ Sor} - 1.805 \text{ Glu} + 0.806 \text{ Fru} - 0.393 \text{ Sucr} - 0.054 \delta^2\text{H} - 1.744$	80.0	
BHB-LP	BHB = $0.847 \text{ Glu} - 1.375 \text{ Fru} + 0.998 \text{ Sucr} + 0.927 \delta^2\text{H} - 1.320$	81.8	80.0
	LP = $-0.514 \text{ Glu} + 0.835 \text{ Fru} - 0.606 \text{ Sucr} - 0.563 \delta^2\text{H} - 0.924$	78.9	
LP-NW	LP = $-0.417 \text{ Sor} + 0.698 \text{ Glu} - 0.886$	68.4	73.5
	NW = $0.508 \text{ Sor} - 0.849 \text{ Glu} - 0.979$	80.0	
BHB-NW	BHB = $-0.594 \text{ Sor} + 2.436 \text{ Glu} - 1.106 \text{ Fru} - 1.609$	81.8	84.6
	NW = $0.439 \text{ Sor} - 1.800 \text{ Glu} + 0.817 \text{ Fru} - 1.193$	86.7	

in fruit [31,32]. Moreover, fruit acidity rather than sweetness was likely to have undergone selection during apple domestication [26]. In this study, it was found that the organic acids were not suitable for distinguishing the Fuji apple samples from these three producing regions. Therefore, it was speculated that organic acids are less affected by temperature, light, and altitude than soluble sugars, which was consistent with the results of correlation analysis of soluble sugars, organic acids, and regional conditions (Table 4).

The fractionation of hydrogen isotopes was affected by distance from the sea, temperature, rainfall, and atmospheric humidity. In this study, BHB (including Hebei Province) is located on the east coast of China and will be affected by coastal climate, so the $\delta^2\text{H}$ value will be different from the samples in inland areas (LP and NW), which can be used to distinguish samples from coastal and inland areas, even from different coasts [10,28]. In addition, the altitude of BHB was lower than LP and NW; these factors will lead to the enrichment of heavy isotopes of hydrogen in BHB samples [33]. Therefore, the decreasing order of $\delta^2\text{H}$ value in samples from the three production regions was BHB (-39.6‰), NW (-44.4‰), and LP (-44.6‰), and the $\delta^2\text{H}$ value in BHB samples was significantly different from LP and NW samples ($P < 0.01$). Oxygen isotopes in plants were affected by latitude, distance from the sea, altitude, and degree of evaporation (mainly affected by humidity and temperature) [34–36]. In addition, in samples in coastal areas, the $\delta^{18}\text{O}$ value was high [37], so it was speculated that the content characteristics of the $\delta^{18}\text{O}$ value in the three production regions were consistent with $\delta^2\text{H}$. The study found that the decreasing order of $\delta^{18}\text{O}$ value was BHB (-4.67‰), NW (-5.22‰), and LP (-5.26‰), and there was a significant difference between BHB and LP samples ($P < 0.05$), but NW samples had no significant difference with BHB and LP samples ($P > 0.05$), possibly because the increase of solar

radiation and the decrease of precipitation in NW lead to the enrichment of oxygen heavy isotopes in samples [36]; furthermore, as the hydrogen isotopes in plants only come from water, the coastal climate had a greater impact on $\delta^2\text{H}$ than $\delta^{18}\text{O}$ [10]. Therefore, the variation in $\delta^{18}\text{O}$ values within NW samples was observed. The $\delta^{13}\text{C}$ value in plants is related to atmospheric photosynthetic carbon sequestration during growth and some environmental factors [38], such as water availability, relative humidity, light intensity, and temperature in the plant environment (atmospheric and soil water) [36,39,40]. In this study, the altitude of NW was higher than LP, and the altitude of BHB was the lowest, and the average $\delta^{13}\text{C}$ value roughly conforms to the characteristics that it increases with the increase of altitude. The average $\delta^{13}\text{C}$ value of samples from high to low was NW (-25.5‰), LP (-25.7‰), and BHB (-26.3‰). This phenomenon was considered to be related to the physiological changes of plants adapting to their growth conditions and the changes of carbon dioxide content with the rise of sea level [41–43].

The fingerprint characteristics of soluble sugars, organic acids, and stable isotopes formed under the influence of geographic and climatic conditions tend to distinguish samples without LP samples, and the correct discrimination rates of the training set were above 82.2%; furthermore, the correct discrimination rates of prediction set were above 80%. The storage time and the sample preparation for detecting sugars and acids in 2017 samples were different from 2018 samples, which may increase the differences in indexes between years (except $\delta^{13}\text{C}$ value); however, the correct discrimination rates of the prediction set were similar to the training set, indicating that although the year may be the main source of variance, there is a variation of soluble sugar, organic acid, and $\delta^2\text{H}$ value, and the discriminant model still has a certain stability. In addition, organic acids were not suitable for distinguishing the

samples from the three main apple production regions, while soluble sugars and stable isotopes were not satisfactorily distinguishing LP and NW samples; therefore, in the following research, it is necessary to add indexes, suitable for distinguishing LP and NW samples, to improve the correct discrimination rate of samples from the three production regions.

5. Conclusions

In this study, as the soluble sugar, organic acid, and stable isotope studied were closely related to the regional conditions, which lead to the poor discrimination rate of LP and NW samples, the correct discrimination rate of the samples from the three main apple production regions was only 69.1%. Nevertheless, it can still be used for many specific applications, such as Glu, Fru, Sucr, and $\delta^2\text{H}$ which can be used to distinguish the samples from the advantageous production areas of apple in China (BHB and LP), and the correct discrimination rates of the training set and prediction set were 82.2% and 80.0%, respectively. In addition, BHB and NW samples can be satisfactorily distinguished by Sor, Glu, and Fru, and the correct discrimination rates of the training set and prediction set were 90.0% and 84.6%, respectively.

Data Availability

Data are included within this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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