Food Chemistry 212 (2016) 367–373

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Combination of the $^{87}Sr/^{86}Sr$ ratio and light stable isotopic values ($\delta^{13}C$, $\delta^{15}N$ and δD) for identifying the geographical origin of winter wheat in China

Hongyan Liu [a], Yimin Wei [a], Hai Lu [b], Shuai Wei [a], Tao Jiang [a], Yingquan Zhang [a], Boli Guo [a,*]

[a] Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences/Comprehensive Key Laboratory of Agro-Products Processing, Ministry of Agriculture, Beijing 100193, China

[b] Division of Chemical Metrology and Analytical Science, National Institute of Metrology, Beijing 100029, China

ARTICLE INFO

Article history:
Received 26 March 2016
Received in revised form 1 June 2016
Accepted 1 June 2016
Available online 2 June 2016

Keywords:
Geographical origin
$\delta^{13}C$
$\delta^{15}N$
δD
$^{87}Sr/^{86}Sr$

ABSTRACT

This study aims to investigate whether isotopic signatures can be used to develop reliable fingerprints for discriminating the geographical origin of Chinese winter wheat, and to evaluate the discrimination effects of $\delta^{13}C$, $\delta^{15}N$ and δD, alone or with $^{87}Sr/^{86}Sr$. In this study, the values of $\delta^{13}C$, $\delta^{15}N$ and δD, and the $^{87}Sr/^{86}Sr$ ratios of wheat and provenance soils from three regions were determined. Significant differences were found in all parameters of wheat and $^{87}Sr/^{86}Sr$ in soil extract (reflecting the bioavailable fraction of soil) among different regions. A significantly positive correlation was observed between the $^{87}Sr/^{86}Sr$ ratios of wheat and soil extracts. An overall correct classification rate of 77.8% was obtained for discriminating wheat from three regions based on light stable isotopes ($\delta^{13}C$, $\delta^{15}N$, and δD). The correct classification rate of 98.1% could be obtained with the combination of the $^{87}Sr/^{86}Sr$ ratio and the light stable isotopic values.

1. Introduction

Wheat is one of worldwide food crops, China plays an important role on worldwide wheat production. The growing area and output of wheat in China are both the largest ones in the world, which account for 1/7 and 17%, respectively (Han, 2015). The quality of agro-product is closely related with its geographical origin that linking with local soil, water and climate and so on (Baroni et al., 2015; Di Paola-Naranjo et al., 2011; Podio et al., 2013). Yellow & Huai valley wheat region is the main production area of winter wheat in China, which plays an important role on China's wheat production and the national food security. In this area, China has constructed some high quality wheat production bases such as *Gaocheng* base in Hebei province and *Yanjin* base in Henan province. The producers of high-quality wheat usually charge a premium more than average (Zhao et al., 2011). However, the genuine wheat is usually replaced with inferior products for financial gain, and the rights of consumers and producers were badly affected.

To protect the consumers against this kind of fraud and to guarantee the rights of producers, effective analytical techniques had been developed to determine the geographical traceability and authenticity of cereal, such as stable isotopic composition (Goitom Asfaha et al., 2011; Wu et al., 2015), multi-element analysis (Cheajesadagul, Arnaudguilhem, Shiowatana, Siripinyanond, & Szpunar, 2013; Li et al., 2012), NIR/IR spectroscopy (Nietner, Pfister, Glomb, & Fauhl-Hassek, 2013; Zhao, Guo, Wei, & Zhang, 2013), and the combination of different techniques (Ariyama, Shinozaki, & Kawasaki, 2012; Podio et al., 2013).

However, discriminate the geographical origin of wheat is never simple, especially for the adjacent wheat productions those share the same climate type, such as the regions in Yellow & Huai valley wheat area, because the differences of isotopic compositions are relatively small and the difficulties of geographical traceability would be enhanced. In terms of the geographical traceability of wheat by stable isotopic ratios, previous studies were focused on the light stable isotopic ratio of $\delta^{13}C$, $\delta^{15}N$, δD, and $\delta^{18}O$ of wheat (Brescia et al., 2002; Luo et al., 2015). However, the discriminant power of $\delta^{13}C$ and $\delta^{18}O$ might be weak when the geographical origins of foods are characterized by a similar climate (Brescia et al., 2002). The combination of stable isotopic fingerprints ($\delta^{13}C$, $\delta^{15}N$, and $^{87}Sr/^{86}Sr$) and elemental profiles achieved good discrimination results for Argentinean wheat (Podio et al., 2013).

$\delta^{13}C$ exhibits a strong ecological effect, $\delta^{13}C$ values of C3 photosynthetic plants are different from those of C4 and CAM plants, and

* Corresponding author.
E-mail address: guoboli2007@126.com (B. Guo).

http://dx.doi.org/10.1016/j.foodchem.2016.06.002
0308-8146/

are also influenced by the climate factors in the growing area of plant that can affect the exchanges of water and CO_2 of the plant with the local environment (Brescia et al., 2002). $\delta^{15}N$ values are based on the nitrogen origins of plants such as soil, manure and chemical fertilizer, which provide information about regional agricultural practices (Högberg, 1997). The variation of δD is closely related to geological factors because δD in rainwater decreases from low latitude to high latitude, from low altitude to high altitude, and from coast to inland (Anderson, 2011; Araguas-Araguas, Froehlich, & Rozanski, 2000). In the meanwhile, climate factors such as precipitation and air temperature will also influence the values of δD in agro-products (Martin & Martin, 2003).

Strontium has four naturally stable isotopes: ^{84}Sr, ^{86}Sr, ^{88}Sr, and ^{87}Sr. The relative proportions of ^{84}Sr, ^{86}Sr, and ^{88}Sr are relatively constant, and the relative proportion of ^{87}Sr was gradually increased with the radioactive decay of ^{87}Rb. The isotopic composition of strontium ($^{87}Sr/^{86}Sr$) is not changed or fractionated by biological processes during strontium transport through the ecosystem because the mass differences among the four strontium isotopes are relatively small (Li, He, Peng, & Jin, 2013). The $^{87}Sr/^{86}Sr$ ratio of plant tissues may be directly related to the cations taken up from the soil, since the isotopic fractionation during this process is assumed to be very small, and anyway it is corrected through a normalization procedure during analysis (Petrini et al., 2015). In recent decades, $^{87}Sr/^{86}Sr$ was successfully applied to identify the geographical origins of vegetables (Swoboda et al., 2008; Trincherini, Baffi, Barbero, Pizzoglio, & Spalla, 2014), beef (Rummel et al., 2012), olive oil (Medini, Janin, Verdoux, & Techer, 2015), wine (Petrini et al., 2015) and other agro-products.

In the study, we investigated the geographic features of light stable isotopes of $\delta^{13}C$, $\delta^{15}N$, and δD and the heavy isotopic ratio of $^{87}Sr/^{86}Sr$ in wheat from different regions, and the relationship of $^{87}Sr/^{86}Sr$ between wheat and soil NH_4NO_3 extracts of different depths, and assessed the discriminant power of light and heavy stable isotopic fingerprints for geographic traceability of winter wheat. The study provided the basis for the identification of geographical origins of wheat and other agro-products in China.

2. Materials and methods

2.1. Sampling and pretreatment

2.1.1. Wheat

Three genotypes of Han 6172, Heng 5229, Zhoumai 16 were selected from three regions of Xinxiang (Henan province), Yangling (Shaanxi province) and Shijiazhuang (Hebei province), which are the most important winter wheat planting regions in China (Fig. 1). Totally 54 wheat samples were collected from three regions in two years of 2013 and 2014, each genotype with three samples. Different genotypes were considered because they had significant influence on the variations of $\delta^{13}C$, $\delta^{15}N$, and δD in agro-product according to previous reports (Araus, Cabrera-Bosquet, Serret, Bort, & Nieto-Taladriz, 2013; Gómez-Alonso & García-Romero, 2009; Liu et al., 2015), we want to test whether the stable isotopic variations among regions are consistent for all the three genotypes of wheat. The altitude ranges from 40 m (above sea level) in Shijiazhuang to 81 m (above sea level) in Xinxiang and 511 m (above sea level) in Yangling. The locally recommended agricultural practices including seeding rates, irrigation methods, fertilization, and chemical control of weeds, pests, and diseases were adopted in each sampling site. Basically, wheat in Xinxiang, Yangling and Shijiazhuang respectively were applied 183 kg/ha, 300 kg/ha and 150 kg/ha urea, and 255 kg/ha, 300 kg/ha and 300 kg/ha diammonium phosphate (DAP) as basic fertilizers, then 255 kg/ha and 210 kg/ha urea were respectively applied as additional fertilizers in Xinxiang and Shijiazhuang, 5 kg/ha monopotassium phosphate was applied as foliar fertilizer in Yangling in March. The climate in Xinxiang and Shijiazhuang is temperate monsoon climate, whereas the climate in Yangling is temperate continental climate. The mean temperatures of Xinxiang, Yangling and Shijiazhuang were, respectively, 10.5 °C, 10.6 °C, and 8.4 °C in the growing season of 2012/2013 and 11.6 °C, 10.3 °C, and 10.2 °C in the growing season of 2013/2014. Wheat kernels were washed quickly with deionized water after picking out stones, weeds, clod, etc., in order to eliminate surface contaminants induced by drying process in the farmers' yards and long-distance transport. Then dried in an oven (DHG-9140A, Yiheng, China) at 38 °C till constant weight, and milled using a miller with a mesh size of 0.075 mm (Cyclotec 1093, Foss Tecator, Denmark).

2.1.2. Soils

In Xinxiang, Yangling and Shijiazhuang, the strata are quaternary holocene, the quaternary upper pleistocene and the paleozoic sediments, respectively, and the soil types are clay, brunisolic soil and loam, respectively. Totally 54 soil samples of three depths (0–20 cm, 20–40 cm and 40–60 cm) were collected, three samples at each soil depth in each region of each year. After residue plant and stones were removed, soil samples were air-dried and then sieved through a 2-mm sieve.

2.2. Analysis of the stable carbon and nitrogen isotopic values

Dry samples of wheat (5–6 mg) were weighed in tin capsules and were then placed in an elemental analyzer (vario PYRO cube, Elementar Company, Germany) with an auto sampler. The elements of the carbon and nitrogen in samples were converted into CO_2 and NO_x gas via combustion at 1020 °C, and then the NO_x was reduced to N_2 with copper wires at 600 °C and entered an isotope ratio mass spectrometer (IsoPrime100, Isoprime Company, Britain) through a Conflo III dilutor. The stable isotope compositions of C and N in each sample were determined according to the same analysis method. Gas (CO_2 and N_2) was calibrated with USGS24 ($\delta^{13}C_{PDB} = -16‰$) and IAEA N_1 ($\delta^{15}N_{air} = 0.4‰$). The values of $\delta^{13}C$ and $\delta^{15}N$ were expressed in delta (δ) notation in parts per thousands (‰) relative to IAEA 600 ($\delta^{13}C_{PDB} = -27.5‰$) and USGS43 ($\delta^{14}N_{air} = 8.44‰$), respectively. The analytical precision for both C and N was 0.2‰. The value of $\delta^{13}C$ and $\delta^{15}N$ were expressed in delta (δ) notation in parts per thousand (‰) relative to V-PDB and N_2, respectively. The delta values were calculated as:

$$\delta(‰) = (R_{sample}/R_{standard} - 1) \times 1000,$$

where δ(‰) refers to the values of $\delta^{13}C$ and $\delta^{15}N$, and R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

2.3. Analysis of the stable hydrogen isotopic values

Dry samples of wheat (1–1.5 mg) were weighed into isotope grade silver capsules (6 × 4 mm) and then folded into tiny balls. All weights were recorded. The samples were transferred into elementary analyzer (vario PYRO cube, Elementar Company, Germany) with an automatic sampler after 72 h balancing. After subsequent pyrolysis at 1450 °C, samples produced CO and H_2, which were then transferred into an isotope ratio mass spectrometer (IsoPrime100, Isoprime Company, Britain). The flow rate of carrier gas was 120 mL/min, and the pressure of H_2 was 1.5 bar. The IAEA-CH-7 polyethylene ($\delta D_{V\text{-}SMOW} = -100.3‰$) was chosen as the reference standard material of δD values. The value of δD was expressed in delta (δ) notation in parts per thousand (‰) relative to V-SMOW. The delta values were calculated as:

Fig. 1. The sampling sites of winter wheat in China.

$$\delta D(‰) = (R_{sample}/R_{standard} - 1) \times 1000,$$

where R is the ratio of D/H.

2.4. $^{87}Sr/^{86}Sr$ analysis

2.4.1. Digestion of wheat samples and extraction of soil samples

Wheat samples (0.25–0.35 g) were pretreated with a mixed solution of HNO_3 and H_2O_2 (BV-III grade, Beijing Institute of Chemical Reagents, Beijing, China) and a microwave digestion instrument (CEM MARS Xpress, CEM, Matthews, USA). The digestion procedures were performed in three steps. Firstly, the temperature was increased to 120 °C within 8 min and maintained for 2 min. Secondly, the temperature was increased to 160 °C within 5 min and was maintained for another 5 min. Thirdly, the temperature was increased to 180 °C within 5 min, was maintained for 15 min, and was then cooled. After cooling to room temperature, the solution was transferred to a beaker, diluted with Milli-Q water, and stored for chemical separation. After each mineralization cycle, a washing cycle was performed with 10% HNO_3 to eliminate cross contamination.

The extraction procedure of DIN 19730 (DIN ISO 19730(2009–07)) was used in order to determine the bioavailable strontium in soil. Briefly, 20 g of soil and 50 mL of 1 mol/L NH_4NO_3 were added to an Erlenmeyer flask, shaken at 20 rpm for 2 h at room temperature, and then allowed to settle for 1 h. At the end of the extraction process, each solution was filtered with a 0.45 μm filter (Pall Corporation, MI, USA) into a 50 mL PFA bottle and was stored for chemical separation. After each cycle of extraction, a washing cycle was performed with 10% HNO_3 to eliminate cross contamination.

2.4.2. Chemical separation and determination of the $^{87}Sr/^{86}Sr$ ratio

Samples were prepared in a Class 1000 clean room (National Institute of Metrology, China) for strontium determination. The wheat digestion solution and the extraction solution of the soil samples were dried on the hotplate and then dissolved in 1–2 mL of distilled 8 mol/L HNO_3. The purpose of this step was to convert the solution into 8 mol/L nitric acid medium, so as to conduct the chemical separation by column chromatography. Acid-cleaned glass columns (4–6 mm in diameter) loaded with Sr Spec resin (Eichrom Industries, Darien, IL, USA) were used to isolate Sr from potential interference elements. Before sample loading, the resin was washed with ultrapure water, and then pre-conditioned with 1 mL of 8 mol/L HNO_3 four times. Recovery experiment was conducted by wheat flour (National Research Center for Certified Reference Materials, Beijing, China), the digestion wheat solution was carefully loaded into the column and then major elements and 99.7% of isobaric Rb were effectively removed in the first 12 mL of 8 mol/L HNO_3. The Sr fraction was further eluted with 8 mL of 0.05 mol/L HNO_3, 99.2% of Sr was recovered according to the method.

The Eichrom eluate was dried and re-dissolved by ultrapure water, the purpose of this step is to prevent the effect of NO_3^- on $^{87}Sr/^{86}Sr$ determination. Samples were loaded on single degassed and zone-refined filaments and dried slowly under a 1.5 A current. The strontium isotope ratios were measured using a thermal ionization mass spectrometer (GV instrument) equipped with a multicollector array consisting of five Faraday cups to focus the five isotopes: ^{84}Sr, ^{85}Rb, ^{86}Sr, ^{87}Sr, and ^{88}Sr. Mass fractionation was rectified by normalizing $^{86}Sr/^{88}Sr$ to 0.1194. Long-term replicated measurement of NIST SRM 987 standard showed an average $^{87}Sr/^{86}Sr$ of 0.71020 ± 0.00002 (2sd, n = 47).

3. Results

3.1. $\delta^{13}C$, $\delta^{15}N$, δD, and $^{87}Sr/^{86}Sr$ of wheat from different regions

The mean values and standard deviations (SD) of the isotopic compositions were measured (Table 1). The values of $\delta^{13}C$, $\delta^{15}N$, and δD and the $^{87}Sr/^{86}Sr$ ratios showed highly significant differences among these three wheat production regions ($p < 0.01$). Although the multiple comparison results among different regions were not consistent for different genotypes, the order of geographical origin was unified according to $\delta^{13}C$, $\delta^{15}N$, and δD and the $^{87}Sr/^{86}Sr$ ratios in each genotype, the $\delta^{13}C$ and δD values of wheat samples from Yangling were the highest, but the $\delta^{15}N$ values were the lowest. The $\delta^{15}N$ values and $^{87}Sr/^{86}Sr$ ratios of samples from Shijiazhuang were the highest.

Table 1
$\delta^{13}C$, $\delta^{15}N$, δD and $^{87}Sr/^{86}Sr$ of wheat from different geographical origins in each genotype of each year.

	Year	Genotype	Xinxiang (n = 18)	Yangling (n = 18)	Shijiazhuang (n = 18)
$\delta^{13}C$	2012/2013	Han 6172	-28.08 ± 0.01^{bB}	-26.31 ± 0.03^{aA}	-28.50 ± 0.10^{cC}
		Heng 5229	-28.43 ± 0.02^{bB}	-26.76 ± 0.03^{aA}	-28.47 ± 0.15^{bB}
		Zhoumai 16	-28.15 ± 0.09^{bB}	-26.28 ± 0.24^{aA}	-28.70 ± 0.08^{cC}
	2013/2014	Han 6172	-28.28 ± 0.22^{bB}	-27.46 ± 0.03^{aA}	-28.01 ± 0.10^{bB}
		Heng 5229	-28.53 ± 0.10^{bAB}	-27.58 ± 0.17^{aA}	-28.17 ± 0.29^{bB}
		Zhoumai 16	-28.39 ± 0.12^{bB}	-27.69 ± 0.17^{aA}	-28.13 ± 0.09^{bB}
$\delta^{15}N$	2012/2013	Han 6172	1.29 ± 0.11^{aA}	-3.35 ± 0.25^{cB}	0.69 ± 0.24^{bB}
		Heng 5229	0.84 ± 0.23^{bB}	-3.20 ± 0.39^{cC}	2.74 ± 0.01^{aA}
		Zhoumai 16	0.57 ± 0.06^{bA}	-3.87 ± 0.10^{cB}	0.74 ± 0.07^{aA}
	2013/2014	Han 6172	-1.62 ± 0.10^{bB}	-5.12 ± 0.02^{cC}	0.07 ± 0.31^{aA}
		Heng 5229	-0.83 ± 0.04^{bB}	-4.60 ± 0.20^{cC}	-0.23 ± 0.18^{aA}
		Zhoumai 16	-1.43 ± 0.06^{bB}	-4.99 ± 0.01^{cC}	0.19 ± 0.05^{aA}
δD	2012/2013	Han 6172	-75.68 ± 3.71^{aA}	-64.94 ± 8.37^{aA}	-73.10 ± 1.19^{aA}
		Heng 5229	-74.92 ± 1.78^{bAB}	-66.39 ± 4.13^{aA}	-80.70 ± 1.98^{cB}
		Zhoumai 16	-68.95 ± 2.26^{aA}	-63.89 ± 12.17^{aA}	-75.69 ± 3.55^{aA}
	2013/2014	Han 6172	-54.00 ± 1.30^{bB}	-49.71 ± 2.67^{aA}	-60.03 ± 1.62^{cB}
		Heng 5229	-60.73 ± 4.01^{bB}	-52.22 ± 0.14^{aA}	-61.10 ± 0.97^{bB}
		Zhoumai 16	-51.18 ± 1.08^{bB}	-43.85 ± 1.54^{aA}	-57.65 ± 0.72^{cC}
$^{87}Sr/^{86}Sr$	2012/2013	Han 6172	0.7111 ± 0.0003^{bB}	0.7112 ± 0.0002^{bB}	0.7122 ± 0.0001^{aA}
		Heng 5229	0.7108 ± 0.0001^{bB}	0.7113 ± 0.0009^{bB}	0.7124 ± 0.0000^{aA}
		Zhoumai 16	0.7105 ± 0.0004^{bB}	0.7116 ± 0.0004^{aA}	0.7119 ± 0.0001^{aA}
	2013/2014	Han 6172	0.7110 ± 0.0006^{bB}	0.7114 ± 0.0000^{bAB}	0.7122 ± 0.0000^{aA}
		Heng 5229	0.7113 ± 0.0001^{bB}	0.7114 ± 0.0000^{bB}	0.7127 ± 0.0000^{aA}
		Zhoumai 16	0.7115 ± 0.0001^{bB}	0.7114 ± 0.0000^{bB}	0.7119 ± 0.0001^{aA}

Note: Data in this table are shown by means ± standard deviations, a-c different letters in the same row indicate significant differences ($p < 0.05$), A-C different letters in the same row indicate highly significant differences ($p < 0.01$).

3.2. The $^{87}Sr/^{86}Sr$ ratio of soil extracts from different regions

The isotopic ratios of $^{87}Sr/^{86}Sr$ were presented as the mean and standard deviation (SD) among different regions at each depth (Table 2). Highly significant differences were observed in soil extracts among different regions ($p < 0.01$). The $^{87}Sr/^{86}Sr$ ratio in the soil extracts from Shijiazhuang was the highest among three regions, whereas the ratio in soil extracts from Yangling was the lowest.

3.3. Correlation of $^{87}Sr/^{86}Sr$ between the wheat and soil extracts

The $^{87}Sr/^{86}Sr$ ratio in wheat had a significantly positive correlation with the ratio in the soil extracts of each depth, according to the results of the Pearson correlation analysis ($p < 0.01$), and the $^{87}Sr/^{86}Sr$ ratios in soil extracts were also significantly correlated with each other among the three different depths ($p < 0.01$) (Table 3). The $^{87}Sr/^{86}Sr$ ratio in wheat was the most significantly positively correlated with that in the soil extracts at the depth of 0–20 cm.

3.4. Canonical discriminant analysis for wheat from different geographical origins

Canonical discriminant analysis was performed by two methods: the light isotopes of $\delta^{13}C$, $\delta^{15}N$ and δD (Table 4a) and the combination of $\delta^{13}C$, $\delta^{15}N$, δD and $^{87}Sr/^{86}Sr$ (Table 4b). The total correct classification rates of the two methods were respectively 77.8% and 98.1%, the correct classification rates of Xinxiang and Shijiazhuang were improved after combining with the $^{87}Sr/^{86}Sr$ ratio. Four isotopes ($\delta^{13}C$, $\delta^{15}N$, δD and $^{87}Sr/^{86}Sr$) were adopted to discriminate wheat from three regions. Two canonical discriminant functions were derived with four isotopes ($\delta^{13}C$, $\delta^{15}N$, δD and $^{87}Sr/^{86}Sr$) and explained the 100% of the variance (function 1 explained 91.2% of the total variance, and function 2 explained 8.8%). Function 1 was mainly obtained with $\delta^{13}C$, $\delta^{15}N$, and δD, and Function 2 was the variable $^{87}Sr/^{86}Sr$. The scores plot (Fig. 2) showed good separation results among different regions. Only one sample was misclas-

Table 3
Pearson correlation and P value for the wheat and soil extract $^{87}Sr/^{86}Sr$ ratios.

	Soil extracts (0–20 cm)	Soil extracts (20–40 cm)	Soil extracts (40–60 cm)
Soil extracts (20–40 cm)	0.909** *0.000*		
Soil extracts (40–60 cm)	0.897** *0.000*	0.975** *0.000*	
Wheat	0.736** *0.000*	0.631** *0.007*	0.585* *0.017*

* Indicates significant correlation ($p < 0.05$).
** Indicates highly significant correlation ($p < 0.01$).

Table 2
The $^{87}Sr/^{86}Sr$ ratio of the soil extracts from different geographical origins at each depth in 2012/2013 and 2013/2014.

Year	Soil extracts (cm)	Xinxiang (n = 18)	Yangling (n = 18)	Shijiazhuang (n = 18)
2012/2013	0–20	0.71169 ± 0.00030^{bB}	0.71159 ± 0.00030^{bB}	0.71277 ± 0.00000^{aA}
	20–40	0.71203 ± 0.00023^{bB}	0.71154 ± 0.00014^{cC}	0.71301 ± 0.00001^{aA}
	40–60	0.71218 ± 0.00024^{bB}	0.71175 ± 0.00009^{cB}	0.71306 ± 0.00005^{aA}
2013/2014	0–20	0.71169 ± 0.00012^{bB}	0.71160 ± 0.00018^{bB}	0.71281 ± 0.00011^{aA}
	20–40	0.71174 ± 0.00004^{bB}	0.71164 ± 0.00013^{bB}	0.71300 ± 0.00005^{aA}
	40–60	0.71193 ± 0.00022^{bB}	0.71160 ± 0.00002^{cB}	0.71313 ± 0.00006^{aA}

Note: Data in this table are shown by means ± standard deviations, a-c in the same row indicate significant differences ($p < 0.05$), A-C in the same row indicate highly significant differences ($p < 0.01$).

Table 4a
The classification of wheat from different geographical origins using $\delta^{13}C$, $\delta^{15}N$ and δD.

		Predicted Group Membership			
		Xinxiang	Yangling	Shijiazhuang	Total
Original	Count	12	0	6	18
		0	18	0	18
		6	0	12	18
	%	66.7	100	66.7	77.8
Cross-validated	Count	11	0	7	18
		0	18	0	18
		8	0	10	18
	%	61.1	100	55.6	72.2

Table 4b
The classification of wheat from different geographical origins using $\delta^{13}C$, $\delta^{15}N$, δD and $^{87}Sr/^{86}Sr$.

		Predicted Group Membership			
		Xinxiang	Yangling	Shijiazhuang	Total
Original	Count	17	0	1	18
		0	18	0	18
		0	0	18	18
	%	94.4	100	100	98.1
Cross-validated	Count	17	0	1	18
		0	18	0	18
		0	0	18	18
	%	94.4	100	100	98.1

sified between Shijiazhuang and Xinxiang, suggesting that these four isotopic ratios contained sufficient information to assess the geographical origin of wheat. Fisher's discrimination functions were as follows:

$$\text{Group 1 (Xinxiang)} = -483.613\,\delta^{13}C + 963.773\,\delta^{15}N + 41.966\,\delta D + 5811719.383\,^{87}Sr/^{86}Sr - 2071532.754$$

$$\text{Group 2(Yangling)} = -472.221\,\delta^{13}C + 950.698\,\delta^{15}N + 41.054\,\delta D + 5808999.672\,^{87}Sr/^{86}Sr - 2069367.163$$

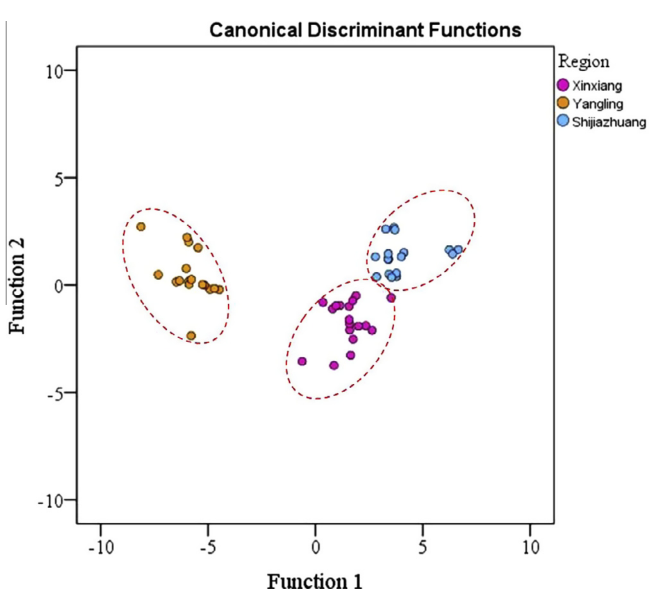

Fig. 2. Discriminant analysis of wheat from different regions based on $\delta^{13}C$, $\delta^{15}N$, δD and $^{87}Sr/^{86}Sr$.

$$\text{Group 3(Shijiazhuang)} = -484.896\,\delta^{13}C + 967.576\,\delta^{15}N + 42.177\,\delta D + 5822399.960\,^{87}Sr/^{86}Sr - 2079156.536$$

4. Discussion

Wheat as C3 plant, the $\delta^{13}C$ could be expressed: $\delta^{13}C_{plant} = \delta^{13}C_{air} - a - (b - a)C_i/C_a$, indicated that the variation of $\delta^{13}C$ in wheat grain was influenced by the $\delta^{13}C$ in air, and the ratio of intercellular CO_2 and CO_2 from the environment (Ci/Ca) (Farquhar, O'leary, & Berry, 1982), and the $\delta^{13}C$ in air rises with latitude (Vaughn et al., 2009). However, Yangling located the lowest latitude among three regions, but the wheat was characterized by the highest values, suggesting that the variation of $\delta^{13}C$ mainly influence by C_i/C_a. Our result was consistent with the previous observation that the $\delta^{13}C$ values in plants increased with altitude (Hobson et al., 2003), higher altitude usually combined with lower atmospheric CO_2 concentration, so we inferred that the change of altitude was mainly responsible for the variation of $\delta^{13}C$ by changing the C_i/C_a.

Synthetic nitrogen fertilizers generally have nitrogen isotope values between −3‰ and 3‰, whereas NO_3^- derived from human and animal wastes contains a higher ^{15}N content, and the $\delta^{15}N$ is between +10% and +20% (McClellan, Vliela, & Michener, 1997). All of the values of $\delta^{15}N$ in wheat were less than 5‰ and were relatively depleted, that was reasonable because the fertilizers (urea and DAP) of three regions were synthetic fertilizers. The variation of $\delta^{15}N$ among different regions might be caused by the applied fertilizers with different nitrate contents (Bateman, Kelly, & Jickells, 2005) and different manufacturers (Bateman & Kelly, 2007). The $\delta^{15}N$ is lower when the amount of synthetic fertilizer applied is higher (Bateman et al., 2005), and the same fertilizer type from different agents also have different $\delta^{15}N$ (Bateman & Kelly, 2007), so the significant lower $\delta^{15}N$ in wheat from Yangling might be result of its higher N inputs and lower $\delta^{15}N$ of its fertilizers. The δD in wheat from three regions decreased in the following order: Yangling > Xinxiang > Shijiazhuang, suggesting that the δD values decreased with the increase in the latitude. The result is in agreement with the previous studies (Heaton, Kelly, Hoogewerff, & Woolfe, 2008).

Although the multiple comparison results of three genotypes were not consistent among different regions, the order of three regions according to the mean values of $\delta^{13}C$, $\delta^{15}N$, δD and $^{87}Sr/^{86}Sr$ ratios in each genotype was unified, these findings could be explained that region contributes more than genotype for the variation of $\delta^{13}C$, $\delta^{15}N$ and δD in wheat, which is in agreement with previous reports (Araus et al., 2013; Liu et al., 2015).

The rock age from three regions decreased in the following order: Xinxiang > Yangling > Shijiazhuang, but the trend of $^{87}Sr/^{86}Sr$ of soil extract and wheat did not obey it, because although the strontium isotope composition of plants and the animals feeding on the plants are related to the $^{87}Sr/^{86}Sr$ signature of the bioavailable strontium derived from water and soil (bedrock) (Baroni et al., 2015), it is also influenced by other Sr sources such as groundwater, rainwater, fertilizers and so on (Sattouf et al., 2007; Vitòria, Otero, Soler, & Canals, 2004), especially the DAP fertilizers applied in the three cities were from different agents and different regions, and the natural abundance of ^{87}Sr in P fertilizer was reported to vary depending on the rock phosphates from which they were produced (Sattouf et al., 2007).

The $^{87}Sr/^{86}Sr$ ratio in the soil extracts (extracted with NH_4NO_3) was positively correlated with the ratio in the wheat in our study. In previous studies, the $^{87}Sr/^{86}Sr$ ratio in the soil extracts was correlated with the $^{87}Sr/^{86}Sr$ ratio in asparagus (Swoboda et al., 2008), wheat (Podio et al., 2013), and grape juice (Durante et al., 2013). To our knowledge, the study of $^{87}Sr/^{86}Sr$ in the wheat grain of China was performed for the first time. The $^{87}Sr/^{86}Sr$ ratios in wheat samples in our study ranged from 0.711 to 0.712. In previous studies, the ranges of $^{87}Sr/^{86}Sr$ ratios in rice samples of China were: 0.710–0.711 (n = 4) (Kawasaki, Oda, & Hirata, 2002) and from 0.708 to 0.713 (n = 50) (Ariyama et al., 2012).

In addition, the Sr isotopic ratios in asparagus corresponded to the ratios obtained from the soils extracted with NH_4NO_3 at a depth of 0–20 cm (Swoboda et al., 2008) and our study highlighted the relationship between wheat and topsoil extracts (0–20 cm). For grape samples, the $^{87}Sr/^{86}Sr$ ratio of the soil extracts at the depth of 40–60 cm was correlated with the $^{87}Sr/^{86}Sr$ ratio of the corresponding grape must (Petrini et al., 2015). The soil depth difference might be interpreted as follows. Most of the root system was concentrated in the upper 40 cm of soil for winter wheat (Zhang, Pei, & Chen, 2004), whereas the most developed depth of grapevine root was 40–60 cm (Petrini et al., 2015).

The correct classification rate obtained based on $\delta^{13}C$, $\delta^{15}N$, and δD was only 77.8% because the isotopic values were similar between Xinxiang and Shijiazhuang. However, the correct classification rate was enhanced significantly by the combination of the $^{87}Sr/^{86}Sr$ ratios and the three light stable isotopic values because the $^{87}Sr/^{86}Sr$ ratios in Shijiazhuang were significantly higher than those in the other two regions. Therefore, both light and heavy isotopic fingerprints should be employed to identify foodstuff from various geographical origins with similar climate or similar geologic background.

5. Conclusion

In our study, the stable isotopic compositions ($\delta^{13}C$, $\delta^{15}N$, δD and $^{87}Sr/^{86}Sr$) of wheat and provenance soils from three regions in the North China Plain were determined. Significant differences were found among different regions for all of the parameters in wheat and for the $^{87}Sr/^{86}Sr$ in soil extracts (extracted by NH_4NO_3), and a significant correlation of $^{87}Sr/^{86}Sr$ was observed between the wheat and soil extracts. A total correct classification rate of 77.8% was achieved using $\delta^{13}C$, $\delta^{15}N$ and δD, and the rate increased to 98.1% when combined with the $^{87}Sr/^{86}Sr$ ratio. In summary, light and heavy isotopes provide complementary information and increase the reliability of the geographical traceability of wheat; further investigation based on a larger scale of samples is required.

Conflict of interest

All authors declare that they have no conflict of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 31371774) and Special Construction of Industrial Technology System for Wheat (No. CARS-03).

References

Anderson, M. A. (2011). Isotopes. In *Encyclopedia of water science*, pp. 684–687). New York: Taylor and Francis.
Araguas-Araguas, L., Froehlich, K., & Rozanski, K. (2000). Deuterium and oxy-18 isotope composition of precipitation and atmospheric moisture. *Hydrological Processes, 14*, 1341–1355.
Araus, J. L., Cabrera-Bosquet, L., Serret, M. D., Bort, J., & Nieto-Taladriz, M. T. (2013). Comparative performance of $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ for phenotyping durum wheat adaptation to a dryland environment. *Functional Plant Biology, 40*, 595.
Ariyama, K., Shinozaki, M., & Kawasaki, A. (2012). Determination of the geographic origin of rice by chemometrics with strontium and lead isotope ratios and multielement concentrations. *Journal of Agricultural and Food Chemistry, 60*(7), 1628–1634.
Baroni, M. V., Podio, N. S., Badini, R. G., Inga, M., Ostera, H. A., Cagnoni, M., ... Wunderlin, D. A. (2015). Linking soil, water, and honey composition to assess the geographical origin of argentinean honey by multielemental and isotopic analyses. *Journal of Agricultural and Food Chemistry, 63*, 4638–4645.
Bateman, A. S., Kelly, S. D., & Jickells, T. D. (2005). Nitrogen isotope relationships between crops and fertilizer implications for using nitrogen isotope analysis as an indicator of agricultural regime. *Journal of Agricultural and Food Chemistry, 53*, 5760–5765.
Bateman, A. S., & Kelly, S. D. (2007). Fertilizer nitrogen isotope signatures. *Isotopes in Environmental and Health Studies, 43*(3), 237–247.
Brescia, M. A., Di Martino, G., Guillou, C., Reniero, F., Sacco, A., & Serra, F. (2002). Differentiation of the geographical origin of durum wheat semolina samples on the basis of isotopic composition. *Rapid Communications in Mass Spectrometry, 16*, 2286–2290.
Cheajesadagul, P., Arnaudguilhem, C., Shiowatana, J., Siripinyanond, A., & Szpunar, J. (2013). Discrimination of geographical origin of rice based on multi-element fingerprinting by high resolution inductively coupled plasma mass spectrometry. *Food Chemistry, 141*, 3504–3509.
Di Paola-Naranjo, R. D., Baroni, M. V., Podio, N. S., Rubinstein, H. R., Fabani, M. P., Badini, R. G., ... Wunderlin, D. A. (2011). Fingerprints for main varieties of argentinean wines: Terroir differentiation by inorganic, organic, and stable isotopic analyses coupled to chemometrics. *Journal of Agricultural and Food Chemistry, 59*(14), 7854–7865.
DIN ISO 19730(2009-07). Soil quality – Extraction of trace elements from soil using ammonium nitrate solution.
Durante, C., Baschieri, C., Bertacchini, L., Cocchi, M., Sighinolfi, S., Silvestri, M., & Marchetti, A. (2013). Geographical traceability based on $^{87}Sr/^{86}Sr$ for PDO Lambrusco wine from Modena. *Food Chemistry, 141*, 2779–2787.
Farquhar, G. D., O'leary, M. H., & Berry, J. A. (1982). On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology, 9*, 121–137.
Goitom Asfaha, D., Quételet, C. R., Thomas, F., Horacek, M., Wimmer, B., Heiss, G., ... Ueckermann, H. (2011). Combining isotopic signatures of n(^{87}Sr)/n(^{86}Sr) and light stable elements (C, N, O, S) with multi-elemental profiling for the authentication of provenance of European cereal samples. *Journal of Cereal Science, 53*(2), 170–177.
GÓmez-Alonso, S., & GarcÍa-Romero, E. (2009). Effect of irrigation and variety on oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) stable isotope composition of grapes cultivated in a warm climate. *Australian Journal of Grape and Wine Research, 16*(2), 283–289.
Han, Y. J. (2015). Wheat trade and production policy research report. In S. H. Xiao et al. (Eds.), *Chinese wheat industry technology development report* (pp. 509–513). Beijing: Chinese Agricultural Press (in Chinese).
Heaton, K., Kelly, S. D., Hoogewerff, J., & Woolfe, M. (2008). Verifying the geographical origin of beef: The application of multi-element isotope and trace element analysis. *Food Chemistry, 107*, 506–515.
Hobson, K. A., Wassenaar, L. I., Mila, B., Lovette, I., Dingle, C., & Smith, T. B. (2003). Stable isotopes as indicators of altitudinal distributions and movements in an Ecuadorean hummingbird community. *Oecologia, 136*, 302–308.
Högberg, P. (1997). Tansley review No. 95 ^{15}N natural abundance in soil-plant systems. *New Phytologist, 137*, 179–203.
Kawasaki, A., Oda, H., & Hirata, T. (2002). Determination of strontium isotope ratio of brown rice for estimating its provenance. *Soil Science and Plant Nutrition, 48*, 635–640.
Li, G., Nunes, L., Wang, Y., Williams, P. N., Zheng, M., Zhang, Q., & Zhu, Y. (2012). Profiling the ionome of rice and its use in discriminating geographical origins at the regional scale, China. *Journal of Environmental Sciences, 25*, 144–154.
Li, Z., He, M., Peng, B., & Jin, Z. (2013). Strontium concentrations and isotope ratios in enamel of healthy and carious teeth in southern Shaanxi, China. *Rapid Communications in Mass Spectrometry, 27*, 1919–1924.
Liu, H., Guo, B., Wei, Y., Wei, S., Ma, Y., & Zhang, W. (2015). Effects of region, genotype, harvest year and their interactions on $\delta^{13}C$, $\delta^{15}N$ and δD in wheat kernels. *Food Chemistry, 171*, 56–61.
Luo, D., Dong, H., Luo, H., Xian, Y., Wan, J., Guo, X., & Wu, Y. (2015). The application of stable isotope ratio analysis to determine the geographical origin of wheat. *Food Chemistry, 174*, 197–201.
Martin, G. J., & Martin, M. L. (2003). Climatic significance of isotope ratios. *Phytochemistry Reviews*, 179–190.
McClellan, J. W., Vliela, I., & Michener, R. H. (1997). Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. *Limnology and Oceanography, 42*, 930–937.
Medini, S., Janin, M., Verdoux, P., & Techer, I. (2015). Methodological development for $^{87}Sr/^{86}Sr$ measurement in olive oil and preliminary discussion of its use for geographical traceability of PDO Nimes (France). *Food Chemistry, 171*, 78–83.
Nietner, T., Pfister, M., Glomb, M. A., & Fauhl-Hassek, C. (2013). Authentication of the botanical and geographical origin of distillers dried grains and solubles (DDGS) by FT-IR spectroscopy. *Journal of Agricultural and Food Chemistry, 61*, 7225–7233.
Petrini, R., Sansone, L., Slejko, F. F., Buccianti, A., Marcuzzo, P., & Tomasi, D. (2015). The $^{87}Sr/^{86}Sr$ strontium isotopic systematics applied to Glera vineyards: A tracer for the geographical origin of the Prosecco. *Food Chemistry, 170*, 138–144.
Podio, N. S., Baroni, M. V., Badini, R. G., Inga, M., Ostera, H. A., Cagnoni, M., ... Wunderlin, D. A. (2013). Elemental and isotopic fingerprint of Argentinean wheat. Matching soil, water, and crop composition to differentiate provenance. *Journal of Agricultural and Food Chemistry, 61*, 3763–3773.

Rummel, S., Dekant, C. H., Hölzl, S., Kelly, S. D., Baxter, M., Marigheto, N., ... Hoogewerff, J. (2012). Sr isotope measurements in beef—analytical challenge and first results. *Analytical & Bioanalytical Chemistry, 402*, 2837–2848.
Sattouf, M., Kratz, S., Diemer, K., Rienitz, O., Fleckenstein, J., Schiel, D., et al. (2007). Identifying the origin of rock phosphates and phosphorus fertilizers through high-precision measurement of the strontium isotopes ^{87}Sr and ^{86}Sr. *Landbauforschung Volkenrode, 57*(1), 2637–2640.
Swoboda, S., Brunner, M., Boulyga, S. F., Galler, P., Horacek, M., & Prohaska, T. (2008). Identification of Marchfeld asparagus using Sr isotope ratio measurements by MC–ICP-MS. *Analytical and Bioanalytical Chemistry, 390*, 487–494.
Trincherini, P. R., Baffi, C., Barbero, P., Pizzoglio, E., & Spalla, S. (2014). Precise determination of strontium isotope ratios by TIMS to authenticate tomato geographical origin. *Food Chemistry, 145*, 349–355.
Vaughn, B. H., Evans, C. U., White, J. W. C., Still, C. J., Masarie, K. A., & Turnbull, J. (2009). Global network measurements of atmospheric trace gas isotopes. In J. B. West, G. J. Bowen, T. E. Dawson, & K. P. Tu (Eds.), *Isoscapes* (pp. 3–31).
Vitòria, L., Otero, N., Soler, A., & Canals, A. (2004). Fertilizer characterization: Isotopic data (N, S, O, C, and Sr). *Environmental Science & Technology, 38*(12), 3254–3262.
Wu, Y., Luo, D., Dong, H., Wan, J., Luo, H., Xian, Y., ... Wang, B. (2015). Geographical origin of cereal grains based on element analyser-stable isotope ratio mass spectrometry (EA-SIRMS). *Food Chemistry, 174*, 553–557.
Zhang, X., Pei, D., & Chen, S. (2004). Root growth and soil water utilization of winter wheat in the North China Plain. *Hydrological Processes, 18*, 2275–2287.
Zhao, H. Y., Guo, B. L., Wei, Y. M., Zhang, B., Sun, S. M., Zhang, L., & Yan, J. H. (2011). Determining the geographic origin of wheat using multielement analysis and multivariate statistics. *Journal of Agricultural and Food Chemistry, 59*, 4397–4402.
Zhao, H., Guo, B., Wei, Y., & Zhang, B. (2013). Near infrared reflectance spectroscopy for determination of the geographical origin of wheat. *Food Chemistry, 138*, 1902–1907.