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An Analytical Method for Detecting the Content of Metallic Elements in Honey Collected from Henan, China: A Systematic Investigation with ICP-AES

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Abstract

In this study, the method for determining ten elements (including K, Na, Ca, Mg, Fe, Mn, Cu, Zn, Pb, and Cd) was designed. With this method, we evaluated 15 honey samples, including three kinds of honey collected from 11 different geographic sites in Henan province of China, with inductively coupled plasma atomic emission spectrometry (ICP-AES). The obtained detecting data were analysed with principal component analysis, correlation analysis, and cluster analysis techniques. The results showed that the recovery is in the range of 93.0−107.0 %, and the relative standard deviations (RSDs) were all below 5.89 %, which indicates that the current analytical method is dependable for the detection of metallic elements in honey.

Keywords

ICP-AES, honey, metallic elements, principal component analysis, cluster analysis

1 Introduction

Honey is produced by honey bees,[1] and contains carbohydrates, metallic elements, and vitamins. Honey could be used to treat burn wounds, stomach disorders and asthma in clinical application, and is a very popular health food because of its nutritional value.[2–4] The content of metallic elements in honey is relatively low (0.1–1 %).[5] The metallic elements in honey mainly include K, Na, Ca, Mg, Cu, Zn, Fe, and Mn, which plays a vital role in maintaining normal metabolic and organ functions, improving the activity of enzymes, and enhancing the immune function.[6–8] The metallic elements in honey come from the plants where the nectar is collected by bees.[9] As a result, the composition and content of metallic elements in honey are not only a sign of its inherent quality, but also a reflection of the environmental conditions of honey plants.

In recent years, the honey industry has been developing rapidly in Henan province, China.[10] In 2016, the total number of bee colonies, the practitioner and the related products in Henan province all ranked well in China.[10–11] The annual production of honey was about a thousand tons, and the output value was almost 1 billion RMB, which accounted for one fifth of the honey industry of the entire country. The quality of honey is evaluated by its density, viscosity, sugar content, and other appearance features.[11] Therefore, the development of a detecting method for metallic elements in honey is important for the honey industry.

In this research, the content of metallic elements in 15 honey samples collected from 11 regions in Henan province of China were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). Principal component analysis and cluster analysis were performed in SPSS software to classify and evaluate the content of metallic elements in these honey samples. We hope the current investigation provides useful information to the further development of honey products. Moreover, the statistical results could give some implication for environmental protection.

2 Experimental

2.1 Materials and reagents

The honey samples were collected from 11 regions in Henan province from May to July 2016. Two to three apiaries were selected in each region. The honey samples include Tung flower honey, Pagoda tree flower honey, and Vitex flower honey, denoted as TFH, PFH, and VFH, respectively. The samples are shown in Table 1.

All chemicals were of guaranteed reagent (GR) including nitric acid, hydrogen peroxide (30 %). The bush branch and leaf used in the research is the National biological standard substance. The standard solutions of K, Na, Ca, Mg, Fe, Mn, Cu, Zn, Pb, and Cd were bought from the PE Company (USA). The water used in the research was the quartz double distilled high pure water.

2.2 Apparatus

The closed intelligent microwave digestion instrument (XT-9912) was bought from Shanghai XTrust Instruments Co. Ltd. A Perkin Elmer Optima 2100 DV inductively coupled plasma atomic emission spectrometer (ICP-AES) was used

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Table 1 – Numbers of honey samples

Number	Sample	Region
S1	TFH	Luyi county, Zhoukou city
S2	TFH	Tuocheng county, Shangqiu city
S3	PFH	Hua county, Anyang city
S4	PFH	Yuanyang county, Xinxiang city
S5	PFH	Lingbao county, Sanmenxia city
S6	PFH	Lushi county, Sanmenxia city
S7	PFH	Wangwu county, Jiyuan city
S8	VFH	Dengfeng city, Zhengzhou city
S9	VFH	Lingbao county, Sanmenxia city
S10	VFH	Linzhou city, Anyang city
S11	VFH	Xinan county, Luoyang city
S12	VFH	Qi county, Hebi city
S13	VFH	Xixia county, Nanyang city
S14	VFH	Yicheng district, Zhumadian city
S15	VFH	Huixian county, Xinxiang city

for measurement. The experimental conditions during measurement were as follows: radio-frequency output was 1.2 kW; flow rate of nebulizer was 0.8 $l\,min^{-1}$; flow rate of cooling was 1.5 $l\,min^{-1}$; sample flow rate was 1.5 $ml\,min^{-1}$.

2.3 Sample preparation

One gram of each honey sample was dissolved in 8 ml concentrated nitric acid and 2 ml hydrogen peroxide in a sealed polytetrafluoroethylene digestion tank. The digestion was conducted as shown in Table 2. After digestion, the system was cooled down to room temperature. The solution from the digestion tank was transferred to a beaker (100 ml), and then heated to remove the acid in the airing chamber. After cooling, the solution was put in a colorimetric tube (10 ml), and diluted with 2 % nitric acid. The blank reagents were prepared according to this procedure for ICP-AES.

Table 2 – Microwave digestion procedure

Stage	Pressure / MPa	Temperature / °C	Time / s	Power / W
1	1.0	130	80	1000
2	1.3	150	200	1200
3	1.8	182	300	1300
4	2.0	200	450	1300

2.4 Data processing

The SPSS 22.0 were used for principal component analysis, correlation analysis, and cluster analysis.

3 Results and discussion

3.1 Effects of the wavelength of the spectral line for each mineral element and the regression analyses

The ICP-AES has the automatic background sync function for correction. It is important to choose the spectral line with low interference, high sensitivity and low background value for the determination of metallic elements in honey. The standard solution was measured using the ICP-AES, and the standard curves were obtained. Measurement was repeated in parallel 10 times. The detection limit was calculated by three times of the standard deviation. The wavelength (λ) of the spectral line, the standard curve, the correlation coefficient (r) and the detection limit (DL) are presented in Table 3. It was obvious that the correlation coefficients of different metallic elements were between 0.9995–0.9999, and the detection limits were between 0.00003–0.01310 $mg\,l^{-1}$, which was suitable for the quantity analysis of the ten kinds of metallic elements.

Table 3 – Analytical spectral lines, linear regression equations, correlation coefficients and detection limits of determination of the metallic elements

Element	Wavelength / nm	Standard curve	Correlation coefficient	Detection limit / $mg\,l^{-1}$
K	766.491	$I = 1903.78c + 635.10$	0.9999	0.00781
Na	589.592	$I = 12658.95c + 1267.60$	0.9998	0.00980
Ca	317.933	$I = 4158.26c - 1532.64$	0.9997	0.01310
Mg	279.079	$I = 12689.15c - 1167.61$	0.9998	0.00103
Fe	238.204	$I = 8591.56c + 67.66$	0.9999	0.00028
Mn	257.610	$I = 30198.32c + 580.91$	0.9996	0.00054
Cu	327.393	$I = 1352.99c + 7.40$	0.9999	0.00038
Zn	206.200	$I = 5980c + 87.06$	0.9999	0.00058
Pb	220.353	$I = 29356c + 201.2$	0.9995	0.00019
Cd	228.802	$I = 485060c + 658.9$	0.9999	0.00003

Table 4 – Recoveries and relative standard deviations (RSD) of ten kinds of metallic elements (n = 6)

Element	Background amount/μg ml^{-1}	Added amount/μg ml^{-1}	Measured amount/μg ml^{-1}	Recovery/%	RSD/%
K	17.130	10.00	27.720	105.9	5.36
Na	4.296	5.00	9.226	98.6	1.89
Ca	2.918	5.00	7.883	99.3	1.27
Mg	0.515	1.00	1.571	105.6	3.26
Fe	0.298	0.50	0.797	99.8	4.65
Mn	0.013	0.10	0.120	107.0	0.84
Cu	0.301	0.50	0.831	106.0	0.28
Zn	0.115	0.10	0.213	98.0	0.39
Pb	0.045	0.10	0.138	93.0	4.89
Cd	0.003	0.10	0.097	94.0	5.89

3.2 Accuracy and precision of the method

In order to assess the accuracy of the method, the standard reference was analysed. The results were compared with the certified ones and the relative error was calculated. The Vitex flower honey from Linzhou city was selected as the standard reference. An amount of 1.0000 g of the standard reference was mixed with the standard solution for measurement. This process was repeated six times to reduce the error, and the mean value was used for the recovery. The relative standard deviation (RSD) is shown in Table 4. The recovery lied between 93.0 and 107.0 %, and the RSD were in the range of 0.28–5.89 % for all metallic elements. With good accuracy and precision, this method could be used for the determination of metallic elements in honey samples.

3.3 Determination of the standard reference material

In order to evaluate the reliability of the method, the bush branch and leaf that is the National biological standard substance was determined. The results were compared with the standard value (Table 5). It was obvious that the measurement results matched the standard values.

Table 5 – Analytical results for standard reference material

Element	Standard value/mg kg^{-1}	Measurement values/mg kg^{-1}	RSD/%
K	8500±500	8943	6.45
Na	11000±1000	10986	8.65
Ca	22200±1300	22900	4.38
Mg	2870±180	2981	2.35
Fe	1020±67	1026	3.30
Mn	58.00±6.00	61.45	2.12
Cu	5.20±0.50	5.63	3.65
Zn	20.60±2.20	21.08	0.51
Pb	7.10±1.10	7.99	0.26
Cd	0.14±0.06	0.15	0.87

3.4 Determination of metallic elements in honey samples

The content of ten kinds of metallic elements were analysed. The measurement was repeated five times, and the results were listed in Table 6. In all these samples, the content of K was the highest, which was consistent with other contributions,[12–13] followed by Na, Ca, Mg, Fe, Cu, Zn, and Mn. The content of harmful heavy metals Pb and Cd were the lowest. The content of K in VFH and PFH was obviously higher than that in TFH. The content of Ca in VFH was distinctly higher than that in PFH and TFH. The content of Fe in TFH was clearly higher than that in VFH and PFH. However, the contents of Na, Mg, Mn, Cu, Zn, Pb, and Cd had little difference in the various kinds of honey samples. It was found that the range of K content (106.40−388.83 mg kg^{-1}) in PFH, the Fe content (4.066−19.861 mg kg^{-1}) in TFH, and the Zn content (1.070−3.45 mg kg^{-1}) in VFH fluctuated. The results showed that the content of the heavy metals Pb and Cd in honey samples from Henan Province were lower than the national standard for food safety[14] and the limit standard of heavy metals of the United Nations Agriculture Organization and the World Health Organization (WHO).[15–16]

Table 6 – Results of the determination of metallic elements in samples

Element	Mass fraction of element/mg kg^{-1}		
	TFH (n = 30)	PFH (n = 75)	VFH (n = 120)
K	41.14±3.85	49.70±2.85	55.46±4.03
Na	172.80±23.01	235.90±31.05	259.72±18.53
Ca	26.15±3.52	22.09±2.58	47.86±4.51
Mg	9.38±3.61	7.66±2.85	7.84±3.12
Fe	10.38±5.25	5.81±2.56	2.95±1.37
Mn	0.11±0.05	0.18±0.14	0.16±0.09
Cu	1.84±0.78	3.01±0.85	2.87±0.85
Zn	2.93±0.84	1.84±0.74	2.06±3.79
Pb	0.65±0.14	0.65±0.19	0.78±0.19
Cd	0.028±0.01	0.035±0.01	0.047±0.01

In conclusion, the contents of metallic elements were different in VFH, PFH, and TFH. Even the same kind of honey from different regions had different contents of metallic elements. As a result, it was necessary to study the characteristics of the metallic elements distribution in honey originating from different plants with the statistical method.

3.5 Characterization of the honey samples

3.5.1 Principal component analysis of the metallic elements

The principal component (PC) analysis was conducted after determining contents of metallic elements, and the results are shown in Table 7. The four PCs accounted for 70.92 %. The characteristic value of the first PC was 2.86, accounting for 28.63 % of the total variance. Among the first PC, Mg, K, and Pb accounted for the most, indicating that these three kinds of metallic elements contributed mostly to the first PC. The second PC accounted for 20.26 % of the total variance with a characteristic value of 2.03. The higher loadings of Ca, Na, Fe, and Mn indicated that these metallic elements had significant relevance to the second PC. The third PC accounted for 12.68 % with a characteristic value of 1.27. The loading of Zn in the third PC was very high. The share and characteristic value of the forth PC were both very low. A proportion of 48.89 % of the total variance came from the first two main components, thus it was believed that the characteristic metallic elements in the honey were Mg, K, Pb, Ca, Na, Fe, and Mn. A plot of PC1 against PC2 (Fig. 1) showed that K, Na, Ca, Mg, Zn, Pb, and Cd were closely correlated with PC1. However, Fe had negative correlation with PC1. Na and Ca were negatively correlated with PC2, and the other metallic elements were positively correlated with PC2.

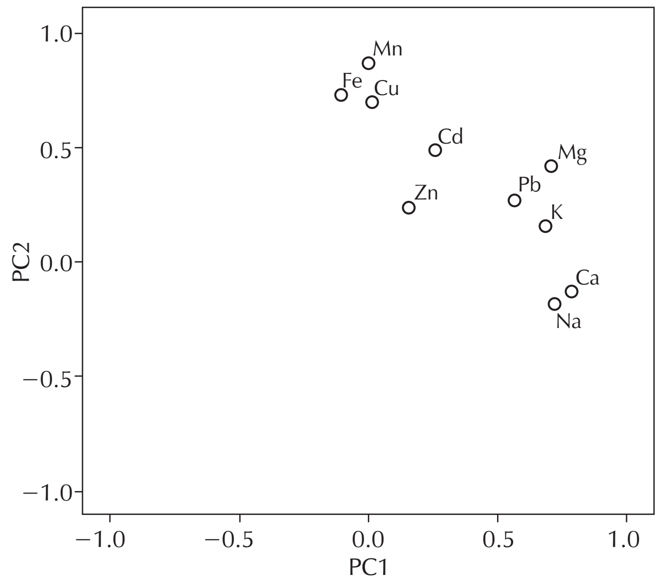

Fig. 1 – Loading plot of the first two principal components for ten metallic elements in honey

3.5.2 Cluster analysis of the metallic elements in honey

The cluster analysis is frequently used to screen data for clustering of samples. The average linkage clustering method and Pearson coefficient clustering method were used. As shown in Fig. 2, the cluster analysis exposed the presence of three distinct groups: one comprising Pb, Cu, Na, Mg, K, Zn, Cd, and Mn; one comprising Ca; and another comprising Fe. The results of cluster analysis were consistent with that of principal component analysis and correlation analysis, indicating that the metallic elements in honey were closely related to the producing of honey and its environment.

Table 7 – Rotated component matrix of PCA results for ten kinds of metallic elements in honey

Mineral	Principal components			
	1	2	3	4
Mg	0.805	−0.145	0.055	−0.124
K	0.619	−0.327	−0.305	−0.096
Pb	0.601	−0.165	0.448	−0.076
Mn	0.569	0.654	0.121	−0.089
Fe	0.394	0.621	0.043	0.332
Ca	0.507	−0.609	0.091	0.090
Na	0.425	−0.606	−0.247	0.452
Cu	0.465	0.523	−0.096	0.468
Zn	0.273	0.080	0.850	−0.111
Cd	0.514	0.199	−0.392	−0.585
characteristic value	2.86	2.03	1.27	0.94
percentage / %	28.63	20.26	12.68	9.35
total percentage / %	28.63	48.89	61.57	70.92

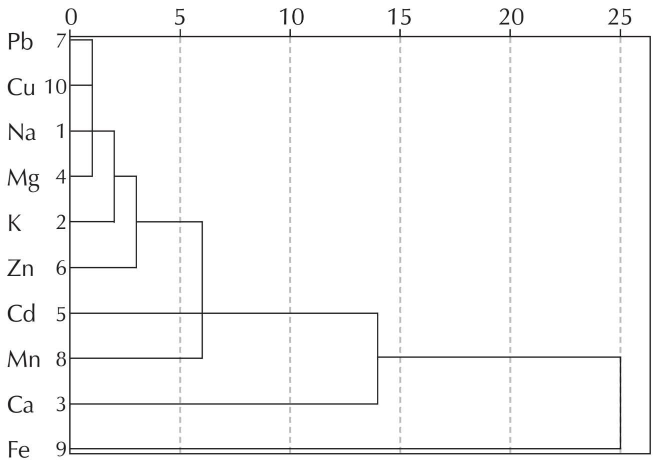

Fig. 2 – Dendrogram of CA for ten elements in honey

3.5.3 Cluster analysis of types of honey

SPSS 22.0 was applied to analyse the three kinds of honey from different regions with ten metallic elements as the variables. The average linkage method was used to connect the two samples. The results of cluster analysis of the different kinds of honey are shown in Fig. 3. The cluster analysis of the different kinds of honey exposed the presence of three distinct groups: one comprising S15; one comprising S1, S2, S3; and another comprising S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14. It was found that the same kind of honey from different regions was not classified into one group. The S1 and S2 in the second group was TFH. The S8, S9, S10, S11, S12, S13, S14 in the third group was VFH, and the S4, S5, S6, S7 in the third group was PFH. The reason may be that the metallic elements in the honey were affected by the geographical and environmental characteristics, leading to different contents of metallic elements in same kind of honey from different areas.

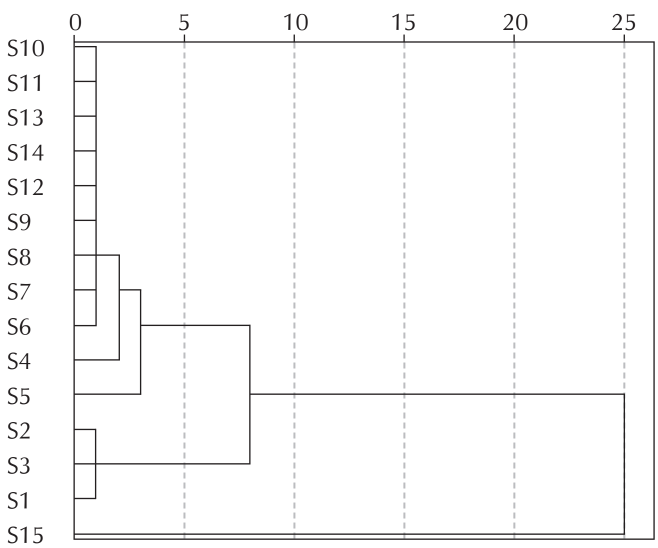

Fig. 3 – Dendrogram of CA for different kinds of honey from Henan province

4 Conclusions

Ten metallic elements in three kinds of honey collected from 11 regions of Henan province were determined by inductively coupled plasma-atomic emission spectrometer (ICP-AES). The contents of metallic elements in honey derived from different plants and regions showed great differences. The content of K was the highest, followed by Na, Ca, Mg, Fe, Cu, Zn, and Mn. The contents of toxic metallic elements Pb and Cd were the least, lower than the national standard of food contaminants (GB2672-2012) and the limitation standards of toxic metals of the WHO.

The principal component analysis suggested that the first four principal components accounted for 70.92 %. Mg, K, Pb, Ca, Na, Fe, and Mn were the characteristic metallic elements in honey, providing theoretical foundation for the research of the health function of honey. The cluster analysis of the metallic elements showed that Pb, Cu, Na, Mg, K, Zn, Cd, Mn was the first cluster; Ca was the second cluster; and Fe was the third cluster. The results of cluster analysis were consistent with that of principal component analysis and correlation analysis. The cluster analysis of the types of honey showed that the same kind of honey from different regions was not classified into one group. The reason may be that the metallic elements in honey were affected by the geographical and environmental conditions of Henan province.

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List of abbreviations and symbols

GR	– guaranteed reagent
ICP-AES	– inductively coupled plasma atomic emission spectrometry
PCs	– principal components
PFH	– Pagoda tree flower honey
RSD	– relative standard deviation, %
TFH	– Tung flower honey
VFH	– Vitex flower honey
WHO	– World Health Organization
r	– correlation coefficient

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SAŽETAK

Metali u medu iz provincije Henan, Kina: sustavna analiza metodom ICP-AES

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U ovom istraživanju osmišljena je metoda određivanja deset elemenata (K, Na, Ca, Mg, Fe, Mn, Cu, Zn, Pb i Cd). Tom metodom evaluirano je 15 uzoraka meda, uključujući tri vrste meda prikupljenih s 11 lokaliteta u provinciji Henan, Kina, atomskom emisijskom spektrometrijom s induktivno spregnutom plazmom (ICP-AES). Dobiveni podatci proučeni su analizom glavnih komponenti, korelacijskom analizom i tehnikama klasterskih analiza. Rezultati su pokazali da se oporaba kreće u rasponu od 93,0 do 107,0 %, a relativne standardne devijacije (RSD) bile su ispod 5,89 %, što ukazuje da je trenutačna analitička metoda pouzdana za otkrivanje metala u medu.

Ključne riječi
ICP-AES, med, metali, analiza glavnih komponenti, klasterska analiza

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