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Authentication of fresh apple juice by stable isotope ratios of δD, $\delta^{18}O$ and $\delta^{13}C$

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Abstract

The authentication of fresh apple juice (FAJ) is a significant issue. However, the main characteristics of FAJ, diluted apple juice (DAJ) with added concentrated apple juice (DAJ+CAJ), and DAJ with added sucrose (DAJ+s) are not different when using classical chemical analyses in juice authentication. In this study, isotope ratio mass spectrometry (IRMS) was used to determine the δD, $\delta^{18}O$, and $\delta^{13}C$ in adulterated apple juice for juice authentication. The δD and $\delta^{18}O$ values were decreased in adulterated juice. A linear correlation was found between δD and $\delta^{18}O$ in apple juices with different amount of adulteration. Therefore, this linear relationship can be used to determine the amount of adulteration that took place. The $\delta^{13}C$ value and the content of each component in real apple juice are within specific ranges. The adulteration can be recognized once the $\delta^{13}C$ value and the content of each component in the apple juice are out of these ranges. The implementation of this method would help maintain the juice market's stability and protect consumers' interests.

Keywords: apple juice; adulteration; isotope ratio mass spectrometry.

Practical Application: This study provides an alternative authentication method for commercial fresh apple juice producers using δD, $\delta^{18}O$, or $\delta^{13}C$.

1 Introduction

Fresh apple juice (FAJ) is produced by pressing and sterilization apple fruits. Currently, FAJ has become a healthy drink and is well accepted by consumers (Clark, 2006; Noci et al., 2008). More than 16 million tons of fruit juice is consumed each year globally. However, FAJ has become a target for adulteration because of its vast market demand and higher price. Water and sugar are typically used in juice adulteration (Jamin et al., 2003). Thus, the authentication of FAJ has been an important issue for government authorities (O'Rourke et al., 2003).

To ensure fair market competition and promote international trade, developing an analytical method to detect fraudulent substitution of FAJ is critical and necessary. Two types of adulterated apple juice (AAJ) are commonly found in the market. Either concentrated juice or sugar was added to the diluted apple juice (DAJ) to maintain the soluble solid content as that of the FAJ (Figure 1). However, the ingredients of FAJ, DAJ with added concentrated apple juice (DAJ+CAJ), and DAJ with added sucrose (DAJ+s) are identical when using traditional chemical analyses in juice authentication (León et al., 2005). The classical chemical identifications are based on the composition and content of chemical components, including soluble solids, sugars, and amino acid nitrogen (Llorente et al., 2011; León et al., 2005; Rødbotten et al., 2009). This identification method is invalid as it cannot differentiate the source of chemical substances in FAJ from that of DAJ+CAJ and DAJ+s.

Recently, isotope ratio mass spectrometry (IRMS) has attracted extensive attention and has become a useful tool for juice authentication (Camin et al., 2015; Ehleringer et al., 2015). Specific isotopic methods have been approved by the American Association of Analytical Chemists (AOAC) and the Committee for Standardization (CEN). Isotope refers to the general name of a class of elements with the same number of protons and different neutron numbers. It can be divided into stable isotopes and radioisotopes. Stable isotope analysis can be used for rapid and accurate identification of adulterated fruit juices. Effective analysis methods for identifying juice authenticity are stable carbon isotope $\delta^{13}C$, hydrogen isotope δD, and oxygen isotope $\delta^{18}O$ ratio mass spectrometry (Bontempo et al., 2014; Camin et al., 2015). The δD and $\delta^{18}O$ values of fresh juice are higher than that of groundwater because transpiration enriches δD and $\delta^{18}O$ of water in the fruit. AAJ has been added water, so it has a lower value of δD and $\delta^{18}O$ (Bizjak Bat et al., 2016; Jezek & Suhaj, 2001; Magdas & Puscas, 2011). As for stable carbon isotope, the difference between common sugar-producing crops (e.g., corn and sugarcane) with C4-type metabolites and some fruits (e.g., apple and coffee) with C3-type metabolites, which results in a higher ^{13}C concentration in fruits compared to sugar-producing crops of C4-type plants (Cabanero et al., 2006; Guyon et al., 2014). Therefore, the stable ^{13}C isotope is used to determine exogenously added sugar in fruit juice, which is further applied for juice authentication. The $\delta D/\delta^{18}O/^{13}C$ isotope authentication method has been used to determine the adulteration in orange juice and lemon juice (Bononi et al., 2016; Simpkins et al., 2000). However, the abundance of the stable isotope is varied in various plant species. The correlation of the stable isotope

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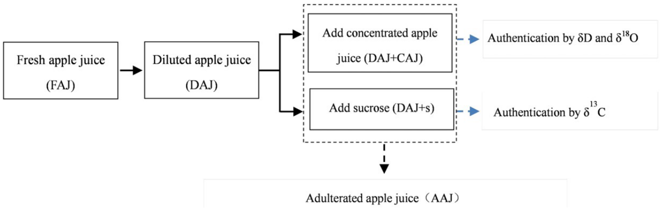


Figure 1. Common fruit juice adulteration process and authentication strategy.

among different species is weak due to the different weather and geography (Dawson et al., 1994; Perini et al., 2018; Wang et al., 2017). Besides, IRMS has not been quantitatively assessed for the degree of adulteration. Thus, the application of the IRMS authentication is still limited.

In this study, isotope ratio mass spectrometry was used to determine the δD, $\delta^{18}O$, and $\delta^{13}C$ in adulterated apple juice. Our study aimed to develop a new and effective identification method by IRMS for adulterated apple juices. Furthermore, this new method could be used to evaluate the degree of adulteration quantitatively and provide a solution for traceability and authenticity of fresh apple juices. This method would help protect the interests of consumers and maintain order in the apple juice market.

2 Materials and methods

2.1 Materials

Thirteen varieties of apples were purchased in Baishui Modern Apple Industry Collaborative Innovation Institute (Xian, China). Thirteen apple varieties were Jiguan (JG), Dailv (DL), Shouhong (SH), Modi (MD), Yuanshuai (YS), Qiaonajin (QNJ), Yanguang (YG), Molishi (MLS), Hanfu (HF), Fuji (FJ), Ziyou (ZY), Gala (GL), and Huashuo (HS). Commercial apple juices used for juice authentication were bought from a local supermarket.

For DAJ+CAJ, the CAJ was first diluted to Brix° that was identical to the soluble solid in fresh apple juice. Then mix diluted CAJ with FAJ. For DAJ+s, the FAJ was mixed with a sugar solution.

2.2 Measurement of C, H, and O isotope ratio

The theory of isotope ratio mass spectrometry (IRMS) is based on the isotope fractionation of light elements (carbon, hydrogen, oxygen, sulfur, nitrogen) with different mass numbers during physical, chemical and biochemical changes. Isotope fractionation will lead to specific changes in the natural abundance of light elements. The theoretical basis of this technology is to use the change of natural abundance of elements to distinguish different geographical origins or varieties of substances. IRMS mainly consists of automatic sampler, element analyzer and mass spectrometer. The pre-treated samples are wrapped in tin cups and entered into the element analyzer through the automatic injector, and the cracking or combustion occurs in the high temperature cracking furnace (H, O) or combustion furnace (C, N). The H and O elements in the sample are converted into H_2 and CO, C through the glass carbon of the cracking tube and N element into CO_2 and N_2 through the combustion furnace. The resulting gases are separated by the gas chromatography column and entered into the isotope mass spectrometer respectively. At the same time, the stable isotope ratios of each element are determined and converted into the isotope abundance values of each element through the processing software. Nowadays, IRMS is widely used in the inspection and identification of honey, fruit juice and wine, as well as in the fields of food origin protection and product traceability.

δD, $\delta^{18}O$ sample preparation and measurement.

δD, $\delta^{18}O$ sample preparation by the micro-distillation method. Samples were thawed at 25 °C before injection analysis. *Isotope analysis*: The measurements of δD and $\delta^{18}O$ in the whole juice were carried out on the Liquid Water Isotope Analyzer (Los Gatos Research, DLT-100, San Jose, USA). Samples of micro-distillation were filtered through the 0.45 μm filter. The deuterium isotope ratios (D/H) were expressed in thousandth fraction (‰). The standard deviation of repeatability of measurements was <0.2‰ for ($^{18}O/^{16}O$) and <0.6‰ for (D/H). All samples were measured under the same laboratory conditions. The isotope ratios of δD and $\delta^{18}O$ were calculated by the thousandth fraction (‰) relative to the Vienna standard mean seawater (VSMOW), with an accuracy of 0.5‰ and 0.15‰, respectively (Equation 1).

$$\delta = \frac{R_{\text{sample}} - R_{\text{VSMOW}}}{R_{\text{VSMOW}}} \times 1000‰ \tag{1}$$

where R_{sample} is the ratio of D/H or $^{18}O/^{16}O$ in the water sample, R_{VSMOW} is the ratio of the standard water sample (VSMOW) D/H or $^{18}O/^{16}O$.

$\delta^{13}C$ sample preparation and measurement

$\delta^{13}C$ sample preparation. We prepared ten samples of FAJ and commercially available juice. Diluted 1:30 and filtered each sample to 2 mL vial. *Isotope analysis:* Isotope analyses of $\delta^{13}C$ are performed on high-performance liquid chromatography linked to isotope ratio mass spectrometry (HPLC-co-IRMS, Thermo Scientific Corporation, Massachusetts, USA); column: carbomix Ca. NP5 (300 mm × 7.8 mm, 5 μm, Sepax Technologies, Delaware, USA);

The $\delta^{13}C$ value of the sample was analyzed and calculated by Isolate 3.0 software (Thermo Scientific, Massachusetts, USA). The $\delta^{13}C$ value was calculated based on the international standard substance Vienna Pee Dee Belemnite standard (VPDB), and the formula is shown below (Equation 2):

$$\delta^{13}\ C = \frac{R_{\text{sample}} - R_{\text{VPDB}}}{R_{\text{VPDB}}} \times 1000‰ \tag{2}$$

where the R_{sample} is the ^{13}C and ^{12}C isotope ratio of the sample, R_{VPDB} = 0. 0112372. The detection limit of HPLC-co-IRMS is 0.7%. While the percentage of the substance is less than 0.7%, the substance is regarded as background.

Time and cost of each analysis

Each analysis cost 300 yuans to determine the adulteration. A juice sample is measured six times, each time around 9-120 seconds.

2.3 Statistical analysis

All charts were completed using Graph Pad Prism 5 (GraphPad Software, California, USA). The data were statistically evaluated using SPSS 18.0 (Chicago, IL). Measurement data are expressed as mean ± standard deviation in all tables. Parametric tests (least significant difference (LSD), ANOVA Analysis of Variance, Pearson correlation test were applied to data because they were normally distributed and fulfilled the assumptions for applying these tests. The percentage of adulteration was used as an independent variable, and δD or $\delta^{18}O$ values as dependent variables for analysis in Figure 2 Supplementary Figure S1 and S2. Take $\delta^{18}O$ as the independent variable and δD as the dependent variable to perform a linear regression analysis in Figure 3. Simultaneously, the linear regression equation and related parameters (r-value, SSE, MSE, and SMSE) were used to analyze the linear relationship of δD and $\delta^{18}O$ under different degrees of adulteration in Table 1.

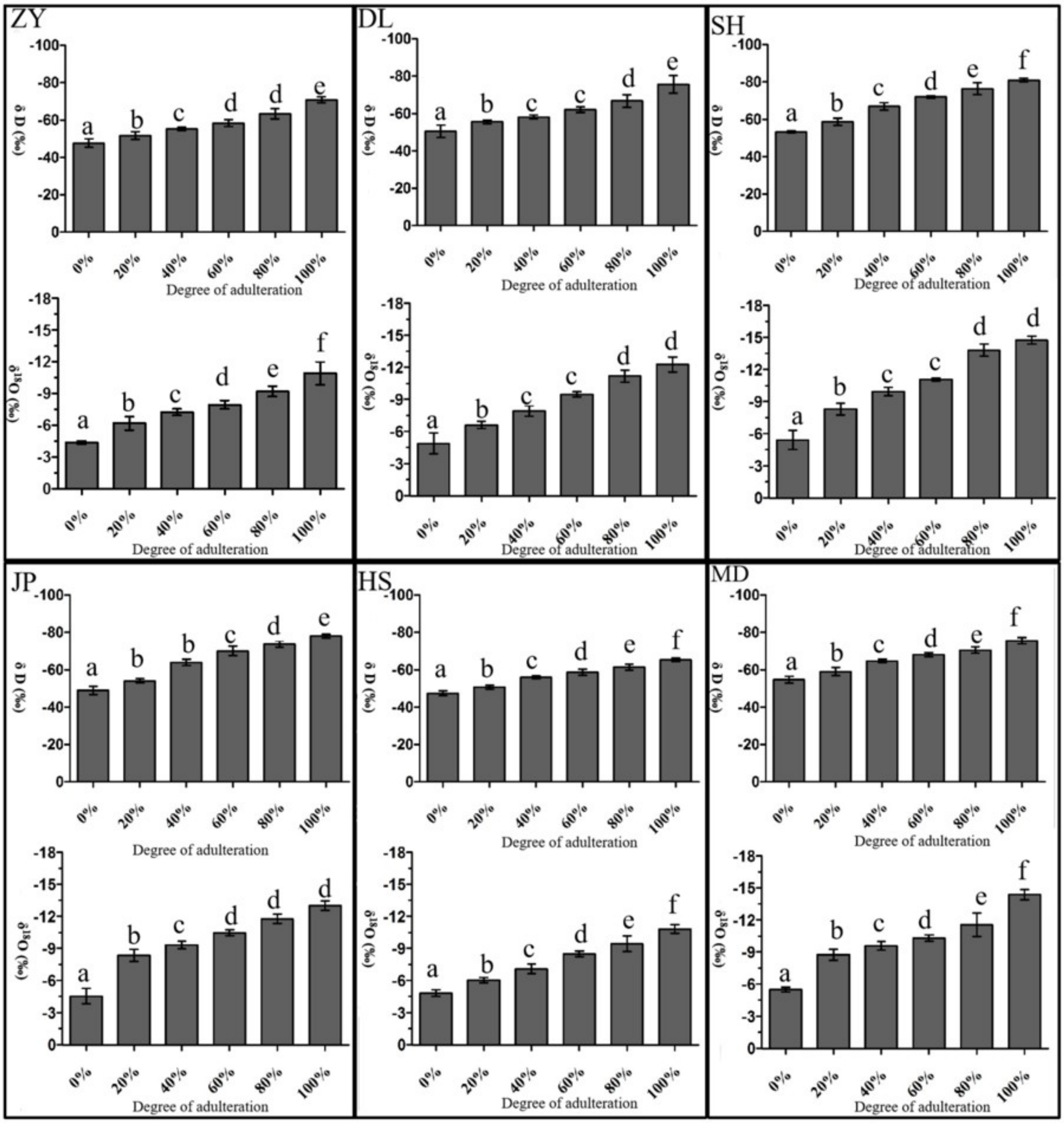


Figure 2. The $\delta^{18}O$ and δD value of six apple juice. 0%, 20%, 40%, 60%, 80%, and 100% represent the amount of sugar or concentration apple juice mixed with fresh apple juice, separately. Error bars represent standard deviations (n=3). Columns with different letters indicate a significant difference ($p<0.05$).

3 Results and discussion

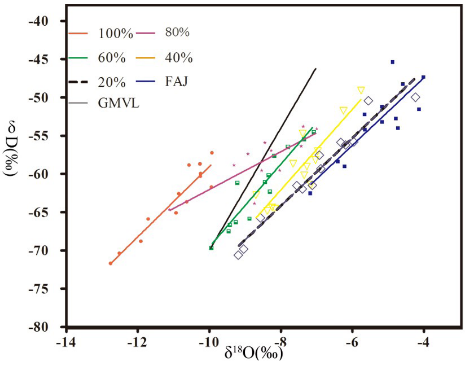
3.1 Authentication analysis of adulterated apple juice counterfeited by concentrated apple juice (DAJ+CAJ)

Characteristics of $\delta^{18}O$ in adulterated apple juice (DAJ+CAJ)

The IRMS was utilized for determining $\delta^{18}O$ in FAJ and DAJ+CAJ. The $\delta^{18}O$ value of thirteen varieties of apple juice was measured (Figure 2, Supplementary Figure S1 and S2). The $\delta^{18}O$ values of DAJ+CAJ are lower than those of FAJ. Although the $\delta^{18}O$ value was lower with the increasing degree of adulteration, the linear relationship between the $\delta^{18}O$ value and the degree of adulteration did not exist. Therefore, the $\delta^{18}O$ variation of DAJ+CAJ with the degree of adulteration needs to be further studied. In addition, the $\delta^{18}O$ values of FAJ ranged from -4.54‰ (JP) to -7.62‰ (JG), which surpass the local tap water (-11.09‰). All the $\delta^{18}O$ values of FAJ were lower than the -4.50‰ $\delta^{18}O$ value of Romanian apple fruit juice (Magdas & Puscas, 2011). Since the geographical environment affects the isotope ratio, there is a difference in the $\delta^{18}O$ of FAJ originated from different growing areas (Bizjak Bat et al., 2016).

Characteristic of δD in adulterated apple juice (DAJ+CAJ)

The δD value of thirteen varieties of apple juice was measured. The δD of all FAJ juices were higher than DAJ+CAJ. The results show that the value of δD varied with the degree of adulteration, but there was no linear relationship between δD value and the degree of adulteration. Therefore, $\delta^{18}O$ or δD can only be used to determine whether the adulteration exists, but not how much adulteration has been done. The δD in fruits is enriched by transpiration, and thus, its value is higher than local underground water where the plant grew. Moreover, the isotope value of a particular species showed some volatility due to the climate environment's influence (Tipple & Pagani, 2013). Furthermore, in Figure 2, Supplementary Figure S1 and S2, the results showed that the δD was distinct from different species, even in the same adulteration degree.



●: 100% adulterated juice, ■: Fresh apple juice, ●: 80% adulterated juice, ■: 60% adulterated juice, ▽: 40% adulterated juice, ◇: 20% adulterated juice.

Figure 3. The linear relationship of $\delta^{18}O$ value between AAJ (DAJ+CAJ) and FAJ in thirteen varieties of apple. GMWL is the global meteoric water line (y=8x+10) (Craig, 1961). GMWL are plotted in each panel for reference. The x-axis is the value of $\delta^{18}O$, and the y-axis is the value of δD.

Relationship between δD and $\delta^{18}O$ in adulterated apple juice (DAJ+CAJ). The isotopic composition of water in plants varies in the different growing periods and different growing environments. However, the relationship between δD and $\delta^{18}O$ is linear correlated. Craig (1961) first found that the δD and $\delta^{18}O$ composition of atmospheric precipitation in North America was linearly correlated (Craig & H., 1961). Based on the experimental data, Craig fitted the equation of the atmospheric precipitation line as (Equation 3):

$$\delta D = 8\delta^{18}O + 10 \tag{3}$$

Using $\delta^{18}O$ as X-axis and δD as Y-axis, the relationship between $\delta^{18}O$ and δD of DAJ+CAJ was linearly fitted in Figure 3. It was interesting that we found a significant correlation between δD and $\delta^{18}O$ with different degrees of adulteration. The regression line of 100% adulteration juice, which was made by water and concentrated juice, is located on the left of GMWL. The regression line of AAJ, which was constituted with FAJ and CJ, is located to the right of GMWL, except for AAJ containing 80% diluted concentrated juice.

Moreover, the higher the degree of adulteration, the farther it was from GMWL. In Figure 3, regression lines of the DAJ+CAJ with different degrees of adulteration were shown. The 60% adulteration was closest to GMWL, and the 20% adulteration was furthest to GMWL. The fitting equation of each regression line was shown separately in Table 1. Six fitting equations has significant difference from 0% adulteration rate ($p = 0.0003$), 20% adulteration rate ($p < 0.0001$), 40% adulteration rate ($p < 0.0001$), 60% adulteration rate ($p < 0.0001$), 80% adulteration rate ($p = 0.0012$) and 100% adulteration rate ($p < 0.0001$) over the sampling periods. Six fitting equations show a good fit from fitting parameters. Thus, both the IRMS method and stable hydrogen

Table 1. The fitting equations between $\delta^{18}O$ and δD value in different degrees of adulterated juice.

Degree of adulteration	Fitting equation	r-value	P-value	SSE	MSE	RMSE
0%	δD_{FAJ} = 4.41 ä^{18}O– 29.78	0.7150	0.0003	78.8531	7.1685	2.6774
20%	$\delta D_{20\%}$ = 4.44 ä^{18}O– 28.64	0.9507	<0.0001	24.4603	2.2237	1.4912
40%	$\delta D_{40\%}$ = 5.27 ä^{18}O– 19.90	0.8144	<0.0001	53.2564	4.8415	2.2003
60%	$\delta D_{60\%}$ =. 5.41 ä^{18}O– 15.43	0.8832	<0.0001	34.7155	3.1560	1.7765
80%	$\delta D_{80\%}$ = 2.47 ä^{18}O– 37.31	0.6304	0.0012	47.0668	4.2788	2.0685
100%	$\delta D_{100\%}$ = 4.60 ä^{18}O– 13.08	0.8883	<0.0001	29.2140	2.6558	1.6297

SSE = the sum of squares due to error; MSE = mean squared error; RMSE = root mean squared error.

isotope δD, and oxygen isotope $\delta^{18}O$ ratio mass spectrometry method are suitable for FAJ adulteration of FAJ.

3.2 Authentication analysis of adulterated apple juice counterfeited by a mixture of fresh juice and sucrose

The different $\delta^{13}C$ values of sugar between FAJ and sugar-producing crops (e.g., corn and sugar cane) are due to either "C3" or "C4" type metabolisms (Guyon et al., 2014). The LC-IRMS was used to detect the carbon stable isotope ratio in fresh apple juice and diluted apple juice mixed with sucrose (DAJ+ sugar).

Characteristic of $\delta^{13}C$ in fresh apple juice

HPLC-IRMS was used to determine $\delta^{13}C$ values of fructose, glucose, disaccharide, and oligosaccharide in FAJ from five apple varieties. And the content of each of these components was calculated using the area normalization method. The data were shown in Table 2. The retention time (RT) of fructose, glucose, disaccharide, and oligosaccharide were 1550.15s, 1126.72s, 950.32s, and 671.37s, respectively (shown in Figure S3). However, the oligosaccharide content was lower than the detection limit in all apple varieties. Therefore, oligosaccharides were regarded as undetected. The $\delta^{13}C$ value of different sugars in FAJ was fructose -25.64~-26.83‰, glucose -25.01~-26.36‰, disaccharide -22.41~-23.23‰ (Table 2).

Moreover, the content of fructose, glucose, and disaccharide were 48.84~52.39%,14.34~28.85%, and 10.47~18.78%, respectively. Based on these, we could deduce that adulteration would occur once the δ13C value and the content of each component in the apple juice are out of these ranges.

Characteristic of $\delta^{13}C$ in adulterated apple juice (DAJ+s)

The LC-IRMS was used to determine the $\delta^{13}C$ value and each component's content in the three adulteration degrees of apple juice (DAJ+s-1, DAJ+s-2, DAJ+s-3). The typical LC_IRMS map was shown in Figure S4, and the data were presented in Table 3. The oligosaccharide content was also lower than the detection limit of 0.7%.

The $\delta^{13}C$ value of different sugar in DAJ+s was fructose -24.91~25.83‰, glucose -22.33~25.33‰, disaccharide -15.94~-21.25‰ (Table 3). So, the $\delta^{13}C$ value of adulterated apple juice was lower than that of fresh juice. Besides, the content of fructose, glucose, and disaccharide were 44.37~52.85%, 19.35~21.70%, and 10.59~23.19%, respectively. Compared to FAJ, the content of the disaccharide component (DAJ+s-1~DAJ+s-3) was lower. Both the value of $\delta^{13}C$ and the content of the disaccharide component were outside the range of FAJ. Therefore, the effectiveness of the IRSM authentication method was proved.

Characteristic of $\delta^{13}C$ in commercial apple juices

Based on the IRSM authentication method, ten different commercial apple juices were purchased from the local market, followed by measuring $\delta^{13}C$ value and each component's content. The typical LC-IRMS chromatogram was shown in Figure S5, and the data were presented in Table 4. The fructose, glucose, and disaccharide $\delta^{13}C$ values in samples-1 are only -19.95‰, -15.66‰ and -14.59‰, which is outside the δ^{13} C range in FAJ (fructose -25.64~-26.83‰, glucose -25.01~-26.36‰, disaccharide -22.41~-23.23‰). As the content of each component, fructose was 44.69%, glucose was 33.00%. Each component did not match the characteristics of FAJ. Therefore, it could be concluded that sample 1 was the adulterated apple juice, which might have added sugar. The fructose and glucose of Sample 2 were within the range of FAJ, and it could be concluded that sample 2 was real apple juice. We could also conclude that samples 3, 4, 5, 6, and 7 were genuinely fresh apple juice, and samples 8, 9, and 10 were adulterated apple juice through our regulation. Therefore, LC-IRMS can be used as a simple and effective method for quality identification of FAJ (Camin et al., 2015)

Table 2. The $\delta^{13}C$ value and percentage of components in fresh apple juice.

Sample	$\delta^{13}C$(‰)			Content(%)		
No.	Fructose	Glucose	Disaccharide	Fructose	Glucose	Disaccharide
FS	-26.83 ± 0.03	-26.36 ± 0.06	-23.23 ± 0.04	51.46 ± 0.11	20.29 ± 0.05	10.47 ± 0.04
ZY	-25.69 ± 0.02	-25.02 ± 0.10	-22.66 ± 0.04	50.06 ± 0.29	16.76 ± 0.18	18.79 ± 0.04
SH	-25.64 ± 0.06	-25.01 ± 0.07	-22.42 ± 0.32	52.39 ± 0.34	21.60 ± 0.06	14.46 ± 0.13
YS	-26.49 ± 0.05	-25.44 ± 0.05	-23.24 ± 0.15	48.84 ± 0.89	14.34 ± 0.25	17.58 ± 0.06
HF	-25.74 ± 0.03	-25.45 ± 0.01	-22.41 ± 0.07	47.91 ± 0.14	28.85 ± 0.12	13.22 ± 0.15

FS, ZY, SH, YS and HF represent the five apple varieties of Fuji, Ziyou, Shouhong, Yuanshuai and Hanfu respectively.

Table 3. The $\delta^{13}C$ value and percentage of components in diluted apple juice with added sugar (DAJ+s).

Sample		$\delta^{13}C$(‰)			Content (%)		
No.		Fructose	Glucose	Disaccharide	Fructose	Glucose	Disaccharide
DAJ+S-1	Observed value	-25.80 ± 0.03	-25.26 ± 0.03	-21.25 ± 0.03	52.85 ± 0.04	21.70 ± 0.10	10.59 ± 0.04
	(Observed value–FAJ valve)	0.00 ± 0.00	0.00 ± 0.00	1.16 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
DAJ+S-2	Observed value	-25.83 ± 0.06	-25.33 ± 0.04	-19.59 ± 0.06	49.75 ± 0.07	20.37 ± 0.05	12.82 ± 0.09
	(Observed value–FAJ valve)	0.00 ± 0.00	0.00 ± 0.00	2.82 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
DAJ+S-3	Observed value	-24.91 ± 0.04	-22.33 ± 0.03	-15.94 ± 0.09	44.37 ± 0.22	19.35 ± 0.14	23.19 ± 0.05
	(Obversed value–FAJ valve)	0.73 ± 0.00	1.77 ± 0.00	6.47 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	4.40 ± 0.01

Table 4. The $\delta^{13}C$ value and percentage of components in commercial apple juice.

Sample		$\delta^{13}C$(‰)			Content(%)		
No.		Fructose	Glucose	Disaccharide	Fructose	Glucose	Disaccharide
Sample-1	Observed value	-19.95 ± 0.06	-15.66 ± 0.05	-14.59 ± 0.07	44.69 ± 0.24	33.00 ± 0.05	15.56 ± 0.13
	(Observed value - FAJ value)	5.69 ± 0.01	9.35 ± 0.01	7.82 ± 0.01	0.00 ± 0.00	4.15 ± 0.00	0.00 ± 0.00
Sample-2	Observed value	-24.08 ± 0.04	-23.94 ± 0.13	-22.21 ± 0.09	57.06 ± 0.98	19.56 ± 0.21	15.33 ± 0.19
	(Observed value – FAJ value)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sample-3	Observed value	-25.62 ± 0.12	-25.63 ± 0.06	-23.43 ± 0.42	50.11 ± 0.11	19.28 ± 0.05	12.78 ± 0.04
	(Observed value – FAJ value)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sample-4	Observed value	-23.34 ± 0.07	-26.02 ± 0.10	-22.16 ± 0.03	49.76 ± 0.09	17.76 ± 0.08	19.12 ± 0.04
	(Observed value – FAJ value)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sample-5	Observed value	-22.44 ± 0.28	-25.65 ± 0.07	-22.42 ± 0.32	51.21 ± 0.34	22.77 ± 0.16	15.25 ± 0.18
	(Observed value – FAJ value)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sample-6	Observed value	-24.53 ± 0.08	-25.71 ± 0.05	-23.34 ± 0.15	48.64 ± 0.72	14.57 ± 0.13	17.46 ± 0.04
	(Observed value – FAJ value)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sample-7	Observed value	-23.74 ± 0.34	-25.43 ± 0.04	-22.11 ± 0.07	49.71 ± 0.15	26.98 ± 0.08	14.47 ± 0.05
	(Observed value – FAJ value)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sample-8	Observed value	-16.95 ± 0.31	-17.42 ± 0.05	-14.45 ± 0.12	35.69 ± 0.19	31.79 ± 0.05	14.98 ± 0.09
	(Observed value – FAJ value)	8.69 ± 0.01	7.59 ± 0.02	8.79 ± 0.01	0.00 ± 0.00	2.94 ± 0.03	0.00 ± 0.00
Sample-9	Observed value	-20.74 ± 0.27	-18.42 ± 0.13	-15.14 ± 0.06	47.69 ± 0.67	35.45 ± 0.05	17.46 ± 0.45
	(Observed value – FAJ value)	4.90 ± 0.01	6.59 ± 0.03	7.27 ± 0.01	0.00 ± 0.00	6.60 ± 0.02	0.00 ± 0.00
Sample-10	Observed value	-17.63 ± 0.14	-16.67 ± 0.21	-13.43 ± 0.04	38.69 ± 0.14	32.76 ± 0.05	16.67 ± 0.13
	(Observed value–FAJ value)	8.01 ± 0.03	8.41 ± 0.02	8.89 ± 0.01	11.86 ± 0.00	8.91 ± 0.01	0.00 ± 0.00

4 Conclusions

This study established an authentication method to identify the adulteration degree of fresh apple juice using δD, $\delta^{18}O$, and $\delta^{13}C$. This method is more efficient and faster than the traditional method. The implementation of this method would contribute to maintaining the stability of the apple juice market and protecting consumers' interests. However, considering the numerous apple varieties and their growing areas, additional in-depth studies on adulterated apple juice are needed in the future.

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Supplementary Material

Supplementary material accompanies this paper.

Figura S1. Os valores de δ18O e δD de seis sucos de maçã. 0%, 20%, 40%, 60%, 80% e 100% representam a quantidade de açúcar ou concentração de suco de maçã misturado ao suco de maçã fresco, separadamente. Barras de erro representam desvios padrão (n = 3). Colunas com letras diferentes indicam diferença significativa (p <0,05).

Figura S2.Os valores de δ18O e δD de um suco de maçã. 0%, 20%, 40%, 60%, 80% e 100% representam a quantidade de açúcar ou concentração de suco de maçã misturado ao suco de maçã fresco, separadamente. Barras de erro representam desvios padrão (n = 3). Colunas com letras diferentes indicam diferença significativa (p <0,05).

Figure S3. LC-IRMS chromatogram of Fresh Apple Juice.

Figure S4. LC-IRMS chromatogram of diluted apple juice+ sugar (DAJ+s).

Figure S5. LC-IRMS chromatogram of commercial apple juice (CAJ)

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