Analytical Methods

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
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Cite this: *Anal. Methods*, 2022, **14**, 4386

Stable isotope ratio analysis of carbon to distinguish sialic acid from freshly stewed bird's nest products†

Di Feng,[abc] Daobing Wang,[ab] Dongliang Wang,[eg] Qiding Zhong,*[abcd] Guohui Li,[ab] Luoqi Zhang,[ab] Nannan Chen,[f] Xiaoxian Lin[eg] and Shu Miao[eg]

Freshly stewed bird's nest products are easily adulterated with exogenous synthetic sialic acid to enhance the grade of the products and sell at high prices. This paper identifies the carbon stable isotope characteristics of sialic acid from natural and commercially synthetic sources using stable isotope ratio mass spectrometry (IRMS). Specifically, an off-line pretreatment technique combined with on-line LC-IRMS was developed to accurately determine $\delta^{13}C$ values of sialic acid in a freshly stewed bird's nest. This method has no obvious isotope fractionation and good reproducibility. EA-IRMS was used to determine the $\delta^{13}C$ values of commercial sialic acid. The results showed that the $\delta^{13}C$ values of sialic acid from natural and synthetic sources were −29.90% ± 0.42% and −16.26% ± 3.91%, respectively, with distinct carbon stable isotope distribution characteristics. By defining a $\delta^{13}C$ threshold value of −28.54% for natural SA, additional commercial SA from a minimum of 10% can be identified. Therefore, $\delta^{13}C$ was proposed as a suitable tool for verifying the authenticity of fresh stewed bird's nests on the market.

Received 17th July 2022
Accepted 14th October 2022

DOI: 10.1039/d2ay01152b

rsc.li/methods

1. Introduction

Edible Bird's nest (EBN), known as Yan Wo in China, is made by golden swiftlets with saliva from their throat glands or a mixture of saliva and down feathers and is a traditional nutritious food.[1,2] China is a major consumer of EBN, with the overall market size of bird's nests reaching 30 billion RMB in China in 2019, with a compound annual growth rate of over 30%, showing a high growth trend.[3] Freshly stewed bird's nest is a popular new product model in recent years. It is made from dried bird's nest steamed at 105 °C ~ 121 °C and canned. The ingredients include dried bird's nest, water and crystal sugar. It has the advantages of "freshness, safety and nutrition" and is very popular among consumers. The amount of dried EBN determines the price of a bottle of freshly stewed bird's nest. Generally speaking, the higher the solid content, the more edible bird's nest is added, and the higher its price is. Therefore, adulteration of bird's nest products always happens.

N-Acetylneuraminic acid (Neu5Ac, also called "sialic acid") is one of the most common and abundant forms in mammals (Fig. 1). The content of sialic acid (SA) in edible bird's nests is the highest among food-derived substances. SA has the characteristics of memory-enhancing,[4] anti-inflammatory[5] and antiviral.[6] The content of sialic acid in an EBN can well reflect the additional amount and quality level of dried EBN in freshly stewed bird's nests and its quality level. It is also a characteristic indicator of the authenticity of freshly stewed bird's nest.[7] In

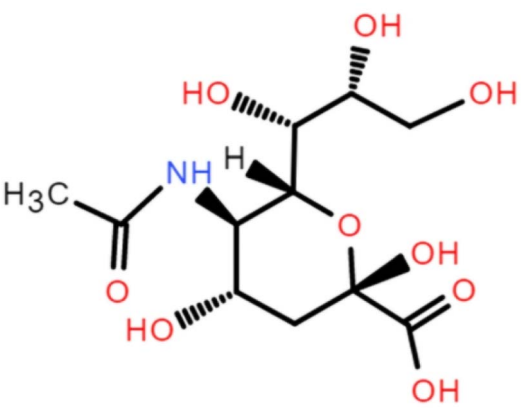

Fig. 1 The structure of sialic acid, also known as *N*-acetylneuraminic acid (Neu5Ac).

[a]*Technology Innovation Center of Light Industrial Consumer Goods Quality and Safety, Beijing 100015, China. E-mail: zhongqiding@163.com*
[b]*Sinolight Technology Innovation Center Co. Ltd, Beijing 100015, China*
[c]*China National Research Institute of Food and Fermentation Industries, Beijing 100015, China*
[d]*Sinolight Inspection & Certification Co., Ltd., Beijing 100016, China*
[e]*Beijing Xiaoxiandun Biotechnology Co., Ltd., Beijing 100020, China*
[f]*Food Industry Promotion Center, Beijing 100015, China*
[g]*Hebei Edible Bird's Nest Fresh Stew Technology Innovation Center, Langfang 065700, China*

† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d2ay01152b

2017, sialic acid was approved as a new ingredient in infant formula.[8–12] The huge price gap between commercial SA ($1 per g) and freshly stewed bird's nest products claiming to contain high content of natural SA ($30 per g) has encouraged adulteration. As the required content of SA in EBN products (0.006 g kg^{-1}–0.560 g kg^{-1}) is regulated,[13] there is a suspicion that freshly stewed bird's nests could be adulterated with purified commercial SA. This is chemically identical to bird's nest SA so undeclared adulteration would lead to a product with a pharmacologically effective SA level.[14]

Several methods have been applied to the adulteration of bird's nest products. Techniques such as sensory evaluation, spectroscopy, mass spectrometry and chromatography have been used to trace the type, source and adulteration of bird's nests using nutrients, physicochemical properties and other characteristics.[15–19] However, the limitation of these methods is that it is impossible to determine whether the SA in bird's nest products is natural or added from commercial sources.

$^{13}C/^{12}C$ is considered a valuable chemical parameter with many applications in food adulteration. In fact, stable carbon isotope analysis provides a powerful tool for tracing the origin and fate of carbon dioxide in the environment.[20,21] Two distinctive features characterise the carbon isotopic composition of organic carbon in plants due to the natural fractionation effect of stable isotopes: firstly, the $^{13}C/^{12}C$ of organic carbon is significantly lower than CO_2 in the air; secondly, different plants have different $^{13}C/^{12}C$ ratios. This is due to plants' thermodynamic effect on carbon isotopes during photosynthesis. Many techniques for determining $\delta^{13}C$ values include Isotope ratio mass spectrometry (IRMS),[22] min-infrared laser spectroscopy[23] and nuclear magnetic resonance (NMR).[24] Of these, IRMS is the preferred method for analysing the carbon isotope ratio at natural abundance due to its advantages, such as relatively high precision (0.1%) and sensitivity (up to 0.01%).[25,26] The development of liquid chromatography coupled with stable carbon isotope ratio mass spectrometry has opened new avenues for analysing isotope carbon ratios in food samples. This is achieved by linking a separation technique to IRMS to enable precise compound-specific isotope analysis (CSIA) at the natural isotopic abundance level.[27]

For the first time in this study, we investigated the possibility of distinguishing commercial and natural SA using LC-IRMS. An off-line pretreatment combined with on-line LC-IRMS was developed to accurately determine $\delta^{13}C$ values of sialic acid in freshly stewed bird's nests. The method was optimised and validated. $\delta^{13}C$ values of commercial pure sialic acid were determined by EA-IRMS. 5 commercial sialic acids and 6 freshly stewed bird's nest products from well-known brands were collected, and these samples were processed and determined. The distribution characteristics of the $\delta^{13}C$ values of sialic acid from different sources were determined. The limited availability of authentic samples from producers and manufacturers of synthetic sources of sialic acid did not allow for establishing an extensive database.

2. Experimental

2.1 Samples

Five commercial SAs with purity >98% were purchased from different manufacturers; SA standard, glucose and fructose, purity >98%, were purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). Beet sugar, purity>98%, was purchased from the manufacturer. Six samples of freshly stewed instant bird's nests (containing sugar) and one sample without sugar were purchased directly from bird's nest companies or certified suppliers, guaranteeing their authenticity. More information on commercial samples of sialic acid and freshly stewed bird's nests can be found in Table S1 and S2.† All of the samples were stored at 4 °C before analysis.

2.2 Materials

Phosphoric acid and sodium persulfate (Sigma-Aldrich, Germany) were analytical reagents used without purification. A carrier gas, helium (BIP grade), and CO_2 (high purity grade) reference gas were produced by Air Products (Beijing, China). Sulfuric acid and sodium hydroxide (analytically pure) were produced by Sinopharm 50 Chemical Reagent Co., Ltd (Beijing, China). Sevage reagent was prepared with a 4 : 1 ratio of chloroform to n-butanol. Chloroform and *n*-butanol were purchased from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Saccharomyces cerevisiae (RW) were purchased in the supermarket (Beijing, China). Water was obtained from a Milli-Q purification system (Millipore, USA).

2.3 Sample preparation

We weighed 50 g of freshly stewed bird's nest after homogenisation, added water to fix the volume to 120 mL and mixed well. Then, *Saccharomyces cerevisiae* activation solution was added and fermented at 30 °C until constant weight. Sulfuric acid was added directly to the fermented sample to give an acid concentration of 0.05 mol l^{-1}. A 100 °C water bath was used for 20 minutes. After removing and cooling to room temperature, it was centrifuged at 6000 r/min for 5 minutes. The supernatant was collected and neutralised with NaOH, concentrated to 30 mL, and then 7 mL Sevage reagent was added and shaken for 25 min. After centrifugation at 6000 rpm for 10 min, the upper layer was collected and repeated until no white residue was produced in the middle layer. The resulting supernatant was evaporated to dryness in a water bath and redissolved in 2 mL of water.

2.4 EA-IRMS analysis

The carbon stable isotope ratios of commercial sialic acid were analysed by an elemental analyser (Flash 2000, Thermo Fisher, Germany) onto a Conflo IV (Thermo Fisher, Germany) interfaced with an isotope ratio mass spectrometry (MAT 253, Thermo Fisher, Germany). A sample of approximately 0.2 mg–0.3 mg was quasi-weighed on an electronic balance and wrapped in a tin cup (Element Microanalysis, USA) for the measurement. The samples were combusted at 960 °C. The reducing tube temperature was 650 °C, and the helium flow rate

was 100 mL min^{-1}. A GC column separated the resulting CO_2 gases at 60 °C.

2.5 LC-IRMS analysis

The $\delta^{13}C$ values of sialic acid extracted from freshly stewed bird's nests were determined by LC-IRMS. The LC system (Thermo Fisher, Germany) was linked to an IRMS instrument (MAT 253, Thermo Fisher, Germany) *via* an IsoLink interface (Thermo Fisher, Germany). A pre-column filter (Chemicals Evaluation and Research Institute, Tokyo, Japan) was installed in front of the analytical column to prevent contamination. The compounds were separated on a Hyper REZ XP carbohydrate H+ (300 mm × 7.7 mm, 8 μm, Thermo Fisher) at 30 °C. The temperature of the interface reactor was set at 99.5 °C. The mobile phase was a 10% H_2SO_4 aqueous solution. The column flow rates were 0.250 mL min^{-1}. The injection volume was 10 μl for all samples. Sodium peroxodisulfate (0.5 M) and phosphoric acid (0.2 M) were mixed and then degassed in an ultrasonic bath for one hour. Phosphoric acid solution and sodium peroxodisulfate solution were used as reaction aids, and the flow rate was 0.05 mL min^{-1} to convert chromatographically separated organics into CO_2. The reagent bottles were degassed with helium during the complete chromatographic run. At the beginning of each run, three pulses of CO_2 reference gas were admitted into the inlet system for about 20 seconds. The constant flow rate during this period gives these peaks a flat-top appearance. A level of CO_2 corresponding to 5 V at m/z 44 was used to calibrate the system. The cycle time for one complete determination was 2500 seconds. Suitable control references were included in each batch. Isodat 3.0 software (Thermo Fisher, Germany) controlled the system.

2.6 Calibration and isotopic calculation

The $^{13}C/^{12}C$ abundance ratio was defined as $\delta^{13}C$ values calibrated against the international standard (Vienna Pee Dee Belemnite, VPDB). The delta notation is defined as

$$\delta^{13}C = [(R_s/R_{st}) - 1] \times 100$$

where R_s is the ratio of $^{13}C/^{12}C$ in the sample and R_{st} is the ratio of the international standard used. The isotopic values were calculated against working standards (SA pure product) and calibrated against international reference materials: caffeine IAEA 600 ($\delta^{13}C_{V\text{-}PDB} = -27.77 \pm 0.04\%$, IAEA-International Atomic Energy Agency, Vienna, Austria) and sugar IAEA–CH–6 ($\delta^{13}C_{V\text{-}PDB} = -10.40 \pm 0.2\%$, IAEA) for $^{13}C/^{12}C$ measurement. Check the $\delta^{13}C$ value of the working reference so that it does not differ from the acceptable value by more than 0.5%. If it did, the spectrometry apparatus settings were checked and adjusted.

3. Results and discussion

3.1 Selection of HPLC columns for the separation of sialic acid from freshly stewed bird's nest

The development of LC-IRMS application methods is challenging due to the content of freshly stewed bird's nest components, the need for a mobile phase free of organic solvents and certain limitations of the system.[28] Therefore, the requirements for the columns are more stringent. As sialic acid is an "acidic sugar", two columns were chosen for comparison that retains polar substances well and is resistant to pure aqueous solutions: the Hyper REZ XP Carbohydrate H+ column (300 mm × 7.7 mm, 8 μm) and the Syncronis aQ (250 mm × 4.6 mm, 5 μm) liquid chromatographic columns. A standard mixture of sialic acid, glucose and fructose was prepared to select a suitable column.

The results of the experiment showed that the Hyper REZ XP Carbohydrate H+ column was more effective in separating sialic acid when the mobile phase was an aqueous sulphuric acid solution at pH = 2, without interference from other peaks (Fig. 2b); the separation of monosaccharides and sialic acid with aQ columns was not effective (Fig. 2a). Thus, the Hyper REZ XP Carbohydrate H+ column was chosen for the separation. The mobile phase ratio, flow rate (0.200 mL min^{-1}–0.400 mL min^{-1}) and column temperature (25 °C–40 °C) were optimised using sialic acid standards. The results showed that the separation of the sialic acid peak was best under the mobile phase of H_2O–H_2SO_4 (90 + 10, v/v), the flow rate of 0.250 mL min^{-1} and column temperature of 30 °C under these chromatographic conditions, SA peaked near 1067 s (Fig. 2b).

3.2 Verification of the effectiveness of anaerobic fermentation for sugar removal

To verify the effectiveness of the fermentation method for sugar removal, we used two commercially available sialic acids with different $\delta^{13}C$ values (−28.17% and −12.91%, respectively). We weighed 1 g accurately in 0%, 25%, 50%, 75% and 100% mixing gradients. The $\delta^{13}C$ values for each mixing gradient were −28.17%, −24.36%, −20.54%, −16.73% and −12.91%, respectively. The prepared 0.01 g mL^{-1} gradient mixture was diluted and determined by LC-IRMS with three parallel measurements per sample. Five gradients mixed scale linear correlation of 0.999 (Fig. 3). Glucose ($\delta^{13}C = -26.25\%$) and gradient mixture

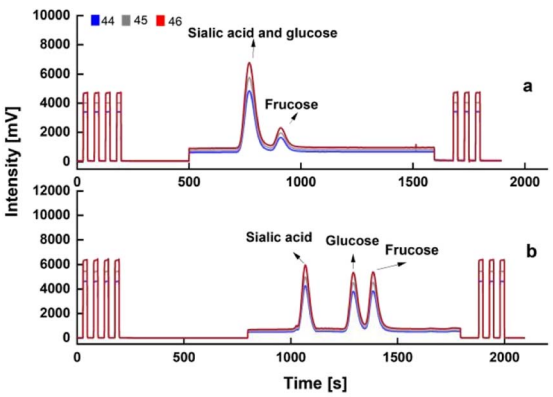

Fig. 2 (a) Syncronis aQ column for separation of sialic acid, glucose and fructose chromatogram; (b) Hyper REZ XP carbohydrate H+ column for on-line separation of sialic acid, glucose and fructose chromatograms.

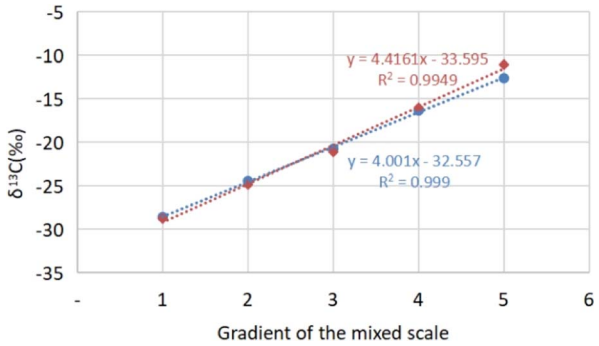

Fig. 3 Two synthetic sources of sialic acid with large differences in carbon values (−28.17% and −12.91%, respectively) were selected for LC-IRMS determination and mixed according to gradients of 0%, 25%, 50%, 75% and 100% ($\delta^{13}C = -28.17\%$, −24.36%, −20.54%, −16.73% and −12.91%, respectively), with $R^2 = 0.999$. LC-IRMS determination of post-fermentation gradient mixing standards with a linear fit $R^2 = 0.9949$.

were selected for simulated fermentation experiments. The specific experimental parameters and data recorded are shown in Table S3.† The experimental results indicate that anaerobic fermentation removes most of the sugars. Then the supernatant after fermentation was rotary evaporated to a certain volume and LC-IRMS determined the $\delta^{13}C$ values of mixed gradients of sialic acid. The results are shown in Fig. 3. The deviations of sialic acid $\delta^{13}C$ values before and after the simulated fermentation were less than 0.25% and the linear fit was good. This indicates that anaerobic fermentation had no significant effect on determining carbon stable isotope values of sialic acid.

Also, the effect of sugar removal by fermentation on determining sialic acid $\delta^{13}C$ values in samples by LC-IRMS was investigated. The fresh stewed sample was selected to determine without treatment and sucrose signal peaks seriously interfere with the accuracy of sialic acid (Fig. 4a). The chromatogram of the freshly stewed bird's nest sample after high-temperature hydrolysis is shown in Fig. 4b. The sucrose in the sample was hydrolysed into glucose and fructose, but the signal

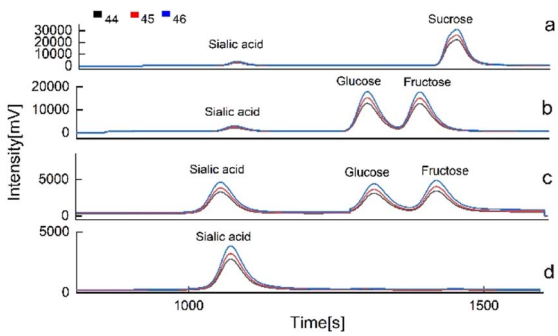

Fig. 4 (a) Chromatogram of freshly stewed bird's nest sample 1 directly into the sample for determination without any treatment; (b) chromatogram of sample hydrolysed at high temperature but not fermented for determination; (c) chromatogram of sample hydrolysed and fermented; (d) chromatogram of fresh stewed bird's nest without sugar.

intensity of both substances was high, which affected the determination of the sialic acid $\delta^{13}C$ value. The chromatogram of the fermented sample is shown in Fig. 4c. In addition, we also measured the $\delta^{13}C$ value of SA in the sample without sugar as a comparison (Fig. 4d). The results showed that the fermentation method can significantly reduce the content of glucose and fructose, and accurately determine the $\delta^{13}C$ value of sialic acid.

In the field of stable isotope technology, ethanol from anaerobic fermentation is commonly to determine the authenticity of products.[29–33] In this study, fermentation was used to reduce the sugar content and ethanol was removed by reduced pressure distillation. The method is economical, convenient and effective to eliminating the interference of bird's nest foaming.

3.3 Validation of the method

To determine the method's reproducibility, the same freshly stewed bird's nest sample was weighed six times and the above pretreatment steps were repeated. The stability coefficients between the carbon isotope ratio determination data of SA were analysed (Table 1). A student's t-test ($p < 0.05$) performed on the data showed no outliers. The standard deviation of repeatability of 0.22% for $\delta^{13}C$ was defined. This is in line with the values found in other matrixes when an extraction step is envisaged.[34]

In this experiment, the high-temperature water bath during sample pretreatment hydrolysed the bound silicic acid to a free state and precipitated some water-soluble proteins. The Sevage method effectively precipitated most of the proteins during the separation and purification of the supernatant.[35] The above pretreatment process was optimised to effectively eliminate matrix interference and alleviate the problem of LC pathway blockage.

The extraction process involved several steps, such as precipitation and centrifugation. If the recovery of SA did not reach 100%, the possibility of isotopic fractionation was considered. Therefore, we verified whether the pretreatment method caused isotopic fractionation. The SA standard product ($\delta^{13}C = -16.67\%$) and beet sugar ($\delta^{13}C = -25.13\%$) were selected for the experiments. 1 g of beet sugar was added to 0.01 g, 0.25 g and 0.5 g of SA, respectively ($n = 3$) and the $\delta^{13}C$ values of sialic

Table 1 The pre-treatment was repeated 6 times on the same fresh stewed bird's nest to check the stability and reproducibility of the method

Number	$\delta^{13}C$% *vs.* V-PDB
1	−29.76
2	−29.91
3	−29.53
4	−30.11
5	−29.68
6	−30.01
Mean	**−29.83**
Std. Dev.	**0.22**

Table 2 Weigh 0.01 g, 0.25 g and 0.5 g of sialic acid standard ($\delta^{13}C = -16.67\%$) to 1 g of beet sugar ($\delta^{13}C = -25.13\%$), respectively, 3 parallel for each sample. Determination after pre-treatment. Verify that isotopic fractionation occurs during the pre-treatment

Number	$\delta^{13}C$ values of SA (%)	Deviation (%)
1	−16.77	0.1
2	−16.60	0.07
3	−16.55	0.15
Mean	**−16.63**	**0.04**
Std. Dev.	**0.13**	**0.13**

acid were measured by LC-IRMS after pretreatment. The results are shown in Table 2. As the differences in the extracted SA were below 0.30%, we concluded that the method did not cause isotopic fractionation.

3.4 $\delta^{13}C$ of commercial and natural SA from fresh stewed bird's nest products

We measured the commercial and natural SA extracted from freshly stewed bird's nest samples based on the established method. As shown in Table 3, natural SA extracted from bird's nests and commercial SA have different $\delta^{13}C$ ranges. Natural SA extracted from authentic samples of freshly stewed bird's nests ranged from −30.63% to −29.50%. There are two main reasons for the distribution of SA $\delta^{13}C$ values in bird's nests: first, some dietary carbon sources have different carbon isotopic signatures; second, the isotopic signatures of foods are absorbed into the tissues of consumers. Birds and other birds of the same genus make nests with saliva or a mixture of saliva and feathers. The saliva produced is affected by factors such as diet and environment.[36] Swiftlets feed on other organisms, including small bees, termites and flying insects;[37] these insects feed primarily on plants that belong to the C3 photosynthetic cycle, with a mean $\delta^{13}C$ of −28.00%. The carbon isotope composition of consumers reflects their diet.[38]

We determined commercially pure sialic acid $\delta^{13}C$ values using EA-IRMS and LC-IRMS. The deviations of the measurements for samples >98% purity were within 0.7%, indicating the high purity of sialic acid. In contrast to SA from bird's nest, commercial SA $\delta^{13}C$ values ranged from −22.77% to −12.01%. To understand why commercial SA behaves the way it does, it is necessary to understand the procedures used to produce it.

Commercial sialic acid is synthesised by chemical, microbial fermentation and enzymatic methods. Most of the commercially available SAs are of fermented origin. The advantage of microbial fermentation for synthesising SA compared to other methods is its low cost. Many studies have focused on cultivation regimes for producing SA. Typically, commercial SA is often produced through fermentation with cheap sorbitol, glycerol and glucose as carbon sources and corn pulp, peptone and yeast paste as nitrogen sources.[39] Combining these carbon and nitrogen sources facilitates the production of their precursors needed, such as pyruvate and *N*-acetylmannosamine, for SA synthesis. Of the ingredients, fermentation substrates contain C3 and C4 origin plants, as there is no standard method for fermentation production of SA; this resulted in $\delta^{13}C$ values between C3 and C4 for different manufacturers of sialic acid.

The $\delta^{13}C$ values of the two enzymatically synthesised samples were −22.77% and −16.59%, respectively. The enzymatic synthesis of sialic acid is more costly than the fermentation method. The enzymatic synthesis is based on the enzyme-catalyzed reaction of *N*-acetylglucosamine as a raw material to synthesise sialic acid.[40] A sialic acid solid drink was also determined to have a $\delta^{13}C$ value of −14.24%. The sialic acid was tentatively determined to be of fermented origin as there is no standard for each synthesis method yet. Thus, there may be significant differences in their $\delta^{13}C$ values for the same synthesis method.

In conclusion, $\delta^{13}C$ values could identify natural and commercial SAs (Fig. 5).

3.5 Authenticity limits

For $\delta^{13}C$, considering a probability level of 95% (mean ± t student × std. dev), a threshold value of −29.90% can be identified for authentic sialic acid. Higher values indicate the presence of commercial SA in the sample.

To quantify the percentage of commercial sialic acid addition to freshly stewed bird's nest, a graph was created based on the mean and the standard deviation (multiplied by *t*-student)

Table 3 LC-IRMS determination of natural salivary acid $\delta^{13}C$ values. EA-IRMS and LC-IRMS results for commercial sialic acid $\delta^{13}C$ values are shown separately. The mean and standard deviation, minimum and maximum values and 95% variability (mean ± Std. Dev) of the different sources of sialic acid are shown

Sample	$\delta^{13}C$ (%, *vs.* V-PDB)		Sample	$\delta^{13}C$ (%, *vs.* V-PDB)
Commercial sialic acid	EA-IRMS	LC-IRMS	Sialic acid from freshly stewed bird's nest	LC-IRMS
1	−17.79	−17.43	1	−29.50
2	−12.91	−13.57	2	−29.54
3	−12.01	−12.41	3	−30.63
4	−22.77	−23.23	4	−29.73
5	−16.59	−16.67	5	−30.12
N-Acetylneuraminic acid solid drink	−25.64	−14.24	6	−29.88
Mean of Sialic acid	**−17.95**	**−16.26**	**Mean of Sialic acid**	**−29.90**
St. Dev. Of sialic acid	**5.38**	**3.91**	**St. Dev. of sialic acid**	**0.42**

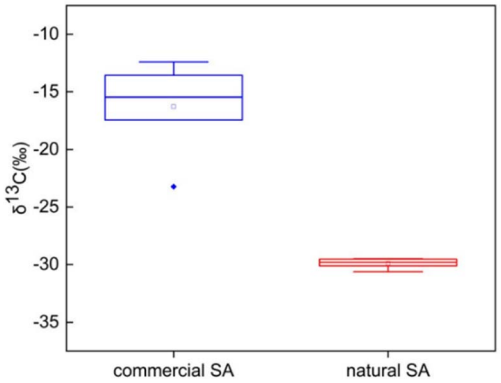

Fig. 5 Box plots of the distribution of $\delta^{13}C$ values of commercial sialic acid and natural sialic acid derived from bird's nest products. The small unfilled squares are sample means and the small blue square is an outlier. The large red and blue boxes are the quartiles. The lowest and highest horizontal lines are the minimum and maximum values.

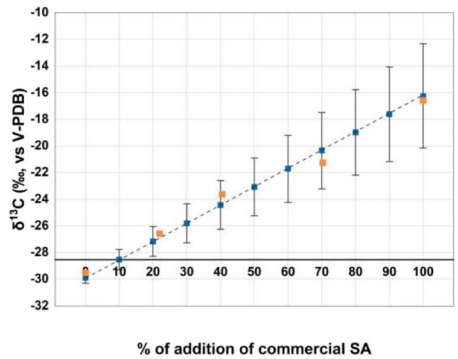

Fig. 6 Graphs of $\delta^{13}C$ values of sialic acid in hypothetical mixtures of freshly stewed bird's nest samples to detect the minimal percentage of commercial sialic acid in a mix. Percentage of natural sialic acid = 100 − % of commercial sialic acid. A broken line defines the threshold limit for natural sialic acid. Blue square: mean value. Bars: 95% confidence limit. Orange square: measured values of prepared mixtures.

of the two groups and increasing the percentage addition of commercial sialic acid to bird's nest products from 0% to 100%. The mean values of the mixture were calculated as the sum of the mean values for the two groups, multiplied by the percentage of contribution to the mix. In contrast, the standard deviation was the sum of the std. Dev. of the two groups multiplied by the contribution percentage, according to the law of error propagation in the case of the sum of two or more variables. The validity of the graph was confirmed by analysing five adulterated mixtures of bird's nest products prepared by adding a growing% (from 20% to 70.3%) of commercial SA ($\delta^{13}C = -16.67\%$) to a natural SA ($\delta^{13}C = -29.50\%$). The values of 5 samples are shown as orange squares in Fig. 6.

It is clear that the limit of −28.54% was the minimum possible detection from 10% illegal addition of commercial sialic acid to freshly stewed bird's nest samples. $\delta^{13}C$ analysis can therefore be proposed as a suitable tool for detecting the authenticity of bird's nests on the market.

4. Conclusions

An analytical method was developed to differentiate between sialic acid extracted from freshly stewed bird's nests and commercial synthetic sialic acid. The method included the analysis of $\delta^{13}C$ values using stable isotope ratio mass spectrometry.

Indeed, a method for accurately determining sialic acid $\delta^{13}C$ values in freshly stewed bird's nest samples by LC-IRMS. The samples were fermented, hydrolysed and purified, and the $\delta^{13}C$ values of sialic acid were determined on-line by LC-IRMS. The developed method was stable, without significant isotopic fractionation and met the determination requirements. SA extracted from samples of authentic bird's nests ranged from −30.63% to −29.50%. While commercial sialic acid $\delta^{13}C$ values for the different synthetic pathways determined by EA-IRMS range from −23.23% to −12.41%. The commercial sialic acid solid drink had $\delta^{13}C$ values of −14.23% and it is assumed that the sialic acid in the product was of fermented origin. The method makes it possible to detect the presence of more than 10% commercial sialic acid in the mixture.

The $\delta^{13}C$ analysis can be a suitable tool to detect the authenticity of sialic acid in freshly stewed bird's nest.

Author contributions

Di Feng: methodology, data curation, writing – original draft. Daobing Wang: investigation, methodology, validation, writing – review & editing. Dongliang Wang: investigation, supervision, methodology, writing – review & editing. Qiding Zhong: conceptualization, investigation, supervision, writing – original draft, writing – review & editing. Guohui Li: methodology, writing – review & editing. Luoqi Zhang: methodology, validation. Nannan Chen: investigation, supervision. Xiaoxian Lin: resources, supervision, writing – review & editing. Shu Miao: resources, supervision, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This research was supported by the National Key R & D Program of China (2019YFC1605203), the Beijing Chaoyang District Science and Technology Program (CYGX2104) and the IAEA Research Agreement 24647 of Coordinated Research Project (D52042).

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