IDENTIFICATION OF AN INTERNAL ISOTOPIC REFERENCE COMPOUND IN PALM SUGAR TO IMPROVE THE DETECTION OF CANE SUGAR ADDITION

FINAL REPORT

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1. SUMMARY

A recent investigation carried out by a UK enforcement authority showed adulteration of palm sugar with cheaper refined cane sugar. As a result, this small study was commissioned to identify an internal isotopic reference marker derived from palm sugar to detect adulteration of palm sugar (a C_3 product) with cane sugar (a C_4 product). It was found that palm sugar contained insufficient organic acids (e.g. shikimic, fumaric, oxalic, malic, citric, tartaric, and succinic acid) to be extracted for internal carbon isotope referencing. Therefore the Association of Official Analytical Chemists method for the determination of C_4 sugars in honey [AOAC method 991.41][1] was successfully adapted for palm sugar analysis. Assessment of the carbon stable isotope composition of bulk palm sugar and the extracted protein fraction has shown that exogenous cane or corn derived sugars are being used to adulterate and extend retail products on sale in the UK. Between 50% to 70% of the sugars present in a small selection of retail palm sugar samples, supplied by Worcestershire Scientific Services and purchased locally in Norwich, were derived from cheaper sources of refined sugar such as cane sucrose or high fructose corn syrup and not from the sap of the Palmyra, Date, Sago or Coconut palms. A surveillance exercise of retail palm sugar products could be undertaken on the basis of the developed methodology to assess the extent of adulteration in the UK market place.

2. INTRODUCTION

2.1 Palm sugar production

Palm sugar was originally made from the sugary sap of the Palmyra palm or the date palm. Now it is also made from the sap of the sago and coconut palms and may be sold as "coconut sugar." When the palms are 15 to 20 years old they start flowering and it is only then that they yield the sap from which palm sugar is made. The sap flows when the flower stem is tapped. To concentrate the nectar into solid sugar, the fresh sap is boiled down and evaporated before being poured into bamboo sections to form cylindrical shapes, or into coconut shells so they emerge as large shallow hemispheres, or into small baskets woven of palm leaves[2].

2.2 Palm sugar authenticity

Palm sugar is used extensively in South East Asian cuisine and can only be produced from mature Palm tree sap. Consequently, it is potentially subject to adulteration with cheaper sources of refined sugar such as cane sucrose. Apparently it is common practice to add small amounts of cane sugar to palm sugar during its production[3]. The cane sugar might be used to 'seed' the crystallisation of the palm sugar crystals. The addition of added sugar is declared on some of the retail products e.g. 'Palm sugar, Water, Glucose'.

2.3 Carbon Isotope Composition

According to the literature and a personal communication[3] palm sugar should possess a characteristic C_3 carbon isotope signature typical for carbohydrate derived from plants that utilise the Calvin Photosynthetic pathway to fix carbon dioxide[,4,5,6]. The average $\delta^{13}C$‰ value for C_3 derived sugars is around -25‰ and cane or corn sugars or syrups (including high fructose corn syrup) average around -10‰[1]. The isotopically 'heavier' (less negative) carbon isotope signatures observed for corn and cane result from the Hatch & Slack photosynthetic (C_4) pathway, which does not fractionate atmospheric carbon dioxide to the same extent as the Calvin (C_3) pathway.

2.4. Internal Isotopic Referencing

Generally the application of stable isotope analysis to food authentication requires the compilation of a databank of authentic reference samples to assess the variation in isotopic natural abundance. This can be time consuming, costly and difficult to arrange for foods produced outside the UK. However, in the case of product extension with cheaper ingredients it may be possible to identify a suitable organic compound, often a secondary metabolite, present in the authentic product that can be extracted in sufficient quantities and used as an Internal Isotopic Reference (IIR) for Carbon Stable Isotope Analysis (CSIA). The carbon isotope ratio of the IIR compound is likely to possess a different but highly correlated carbon isotope signature to the component that may be adulterated. This approach has been successfully used to improve the sensitivity and reliability of detecting cane sugar in a number of premium food products. A summary of such methods is provided in Table 1 below.

Table 1: Official and literature cited methods that utilise internal isotopic references to improve the detection of cane or corn derived sugar products in food using carbon stable isotope analysis

Product	Internal Isotopic Reference	Literature citation
Honey	Honey protein	AOAC method 991.41[1]
Maple syrup	L-Malic acid	Tremblay & Paquin (2007)[7]
Fruit juice	Fruit pulp	Rossmann *et al*., (1997)[8]

After high performance liquid chromatographic analysis of the palm sugar samples It was found that it contained insufficient organic acids (e.g. shikimic, fumaric, oxalic, malic, citric, tartaric, and succinic acid) to be extracted for internal carbon isotope referencing. Therefore the Association of Official Analytical Chemists method for the determination of C_4 sugars in honey [AOAC method 991.41] was successfully adapted for palm sugar analysis.

3. PALM SUGAR SAMPLES

Six retail palm sugar samples had previously been submitted to IFR Extra for carbon stable isotope analysis (CSIA) by Worcestershire Scientific Services (Public Analyst), to establish if cane or corn sugar (or derivatives) had been used to extend the product. In addition, two retail palm sugar samples were purchased locally in Norwich.

4. METHODOLOGY

4.1. Bulk sample preparation for Stable Isotope Analysis (SIA)

Samples of palm sugar ranged from semi-solid crystalline to solid samples. Semi solid samples were thoroughly mixed with a spatula before sub-sampling. Three 1mg ± 0.2 mg subsamples were weighed into tin capsules (6 x 4 mm, Elemental Microanalysis, Okehampton, UK).

4.2. Preparation of protein fraction from palm sugar for SIA

20 ml of ultrapure water was added to 10g of homogenised palm sugar in a 50 ml Greiner tube and mixed well. Dissolution of the sugar crystals was aided either by ultrasonication or warming to 80°C in a water bath. The 50% w/v palm sugar solution was poured into a 50ml disposable syringe and filtered through a 0.22um syringe filter into another 50 ml Greiner tube by applying pressure to the syringe plunger.

5 ml 10% w/v Sodium Tungstate Dihydrate ($Na_2WO_4.2H_2O$) solution and 5 ml 0.335M sulphuric acid (H_2SO_4) was added together in a 15ml Greiner tube and mixed. This was immediately added to the filtered palm sugar solution and mixed thoroughly. The 50ml tube was placed in a water bath at ~80°C until a visible floc (suspended precipitate in the form of flakes) formed with clear supernatant (≥ 2 hours). If no visible floc has formed (≥ 1 hour), or the supernatant remains cloudy, 0.335M sulphuric acid should be added in 4 ml increments, and heated repeatedly between additions.

After a stable floc formed, the 50 ml Greiner tube was centrifuged for 20 minutes at 2000 rpm. The protein floc should form a pellet at the bottom of the Greiner tube. The liquid supernatant was carefully decanted and discarded. 40 ml of ultrapure water was added and mixed thoroughly to re-suspend the protein pellet. The tube was centrifuged at 2000 rpm for 20 minutes and the supernatant was discarded as before. Washing, mixing and centrifuging steps were repeated up to 5 more times with ~ 40 ml portions of ultrapure water, with the pellet thoroughly dispersed each time.

4.3. Stable Isotope Analysis

The carbon stable isotope analyses were performed using a Finnigan XP plus Isotope Ratio Mass Spectrometer (Thermo-Finnigan GmbH, Bremen, FRG) coupled to a Costech ECS4010 elemental analyser (Milan, Italy). The uncertainty of measurements was typically ±0.2 ‰ for $\delta^{13}C$. Stable isotope ratios were expressed in 'δ ‰', according to equation 1 below:

$$\delta X\text{‰} = \frac{\left(\frac{X}{Y}\right)_{sample} - \left(\frac{X}{Y}\right)_{standard}}{\left(\frac{X}{Y}\right)_{standard}} \times 1000 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where X and Y represent the heavier and the lighter isotope, respectively. The standard is an international standard, which is V-PDB (Vienna-Pee Dee Belemnite) for $\delta^{13}C$.

5. RESULTS AND DISCUSSION

5.1. Repeatability of protein extraction and Carbon Stable Isotope Analysis (CSIA)

The repeatability of the protein extraction and carbon stable isotope ratio measurement was assessed. Five separate 50% w/v solutions of the palm sugar retail sample from Norwich (PS09080001) were prepared and processed and measured according to Sections 4.1 to 4.3 above. The results of these analyses are presented in Table 2 below. The table shows the mean $\delta^{13}C$‰ value determined from 3 triplicate determinations (n) on each of the five separate preparations of protein. The sample standard deviation [sd (σn-1)] is also shown.

Table 2: Repeatability of protein extraction and triplicate Carbon Stable Isotope Analysis (CSIA) on five separate preparations of retail palm sugar PS09080001.

Sample description	$\delta^{13}C$‰ PDB	sd (σn-1)	n
PS09080001-protein-1	-24.29	0.02	3
PS09080001-protein-2	-24.03	0.01	3
PS09080001-protein-3	-24.00	0.01	3
PS09080001-protein-4	-24.30	0.18	3
PS09080001-protein-5	-24.34	0.07	3
Mean of means	**-24.19**		
sd of means	**0.16**		
n	**5**		

The overall precision of the analyses (0.16‰) demonstrate that the extraction procedure and carbon stable isotope measurement are repeatable and can be used routinely to obtain consistent results for the extracted palm sugar protein fraction.

5.2. Carbon Stable Isotope Analysis of bulk palm sugar and extracted protein fractions

The retail palm sugar samples purchased locally in Norwich and supplied by Worcestershire Scientific Services were processed and measured according to sections 4.1 to 4.3 outlined above. The results obtained from the carbon stable isotope analysis are displayed in Table 3 below. The table shows the mean $\delta^{13}C$‰ value determined and the sample standard deviation sd (σn-1) obtained from 3 triplicate determinations (n) on the bulk product and the extracted protein from each of the eight retail samples. The accuracy of the analyses was assured by normalising the results against International Atomic Energy Agency cane sucrose reference material IAEA-CH-6[9][10] and using Joint Research Centre (Ispra) cane sucrose[11] as a secondary reference for cross validation. All of the standard deviations of the replicate determinations of $\delta^{13}C$‰ were less than 0.2‰ which is our target precision for this analysis.

Table 3: Carbon Stable Isotope Analysis of bulk palm sugar and extracted protein fractions in retail samples purchased locally in Norwich and supplied by Worcestershire Scientific Services.

Retail samples purchased in Norwich

	Protein fraction			*Bulk palm sugar*			
Sample details	**$\delta^{13}C‰_{PDB}$**	**sd (σn-1)**	**n**	**$\delta^{13}C‰_{PDB}$**	**sd (σn-1)**	**n**	**Estimated cane sugar content (%)**
PS09080001	-24.19	0.02	3	-14.04	0.12	3	70
PS09080002	-20.63	0.05	3	-12.99	0.05	3	70

Retail samples supplied by Worcestershire Scientific Services

	Protein fraction			*Bulk palm sugar*			
Sample details	**$\delta^{13}C‰_{PDB}$**	**sd (σn-1)**	**n**	**$\delta^{13}C‰_{PDB}$**	**sd (σn-1)**	**n**	**Estimated cane sugar content (%)**
EF107-03126	-20.72	0.01	3	-13.60	0.19	3	65
EFF07-30413	-21.94	0.01	3	-13.21	0.03	3	71
EFF08-01126	-21.39	0.03	3	-13.77	0.18	3	65
EF108-01127	-21.77	0.03	3	-14.54	0.11	3	60
EF108-01128	-22.98	0.06	3	-14.90	0.11	3	61
EFF08-01721	-21.21	0.05	3	-14.86	0.09	3	55

5.3. Estimation of the C_4 (cane of corn) plant sugar content of the palm sugar samples

The apparent C_4 sugar content of the palm sugar samples was estimated using the formula given in the AOAC official method for honey listed in Table 1 and provided in the excerpt panel below.

Calculate apparent C-4 sugar content as follows:

$$\text{C-4 sugars, \%} = \frac{\delta^{13}C_P - \delta^{13}C_H}{\delta^{13}C_P - (-9.7)} \times 100$$

where $\delta^{13}C_P$ and $\delta^{13}C_H$ are $\delta^{13}C$ values, ‰, for protein and honey, respectively, and −9.7 is the average $\delta^{13}C$ value for corn syrup, ‰. Report negative values from this calculation as 0%. Product is considered to contain significant C-4 sugars (primarily corn or cane) only at or above a value of 7%.

Figure 1 : Extract from AOAC official method for honey [reference 1] formula for calculation of C-4 sugars

The last column of Table 3 shows that between 50% to 70% of the sugars present in the retail samples of palm sugar on sale in the UK are in fact derived from cheaper sources of refined sugar such as cane sucrose of high fructose corn syrup and not from the sap of the Palmyra palm, date, sago or coconut palms.

6. CONCLUSION

The Association of Official Analytical Chemists method for the determination of C_4 sugars in honey [AOAC method 991.41] has been successfully adapted for palm sugar analysis. Assessment of the carbon stable isotope composition of bulk palm sugar and the extracted protein fraction has shown that significant quantities of exogenous cane or corn derived sugars are being used to adulterate and extend retail products on sale in the UK.

7. FUTURE WORK

It is quite clear from the literature and personal communications from Michele Lees that Palm trees use C_3 photosynthesis and the carbon isotope signature of products from Palms, such as sap, should reflect this pathway. However, in order to fully validate the methodology described here samples of authentic palm sugar, sourced directly from the palm tree sap, need to be analysed by carbon isotope analysis. It is possible that this sap could be sourced in the UK from Kew Gardens or another arboretum.

8. REFERENCES

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