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HPLC/EA-IRMS: Identifying adulterated coconut juice using isotope fingerprints

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Goal

Illustrate how carbon isotope fingerprints identify adulteration of commercially available coconut juice.

Introduction

The authenticity of commercially available coconut water is of increasing importance because of its designation as a juice by the European Fruit Juice Association (AIJN) and the increasing consumer perspective that it is a healthy, low-carbohydrate beverage. It has been noted that recent trends in addition of sugar to enhance taste and attractiveness of the coconut juice have resulted in an increased sale, however, opening the possibility to fraudulently mis-label coconut juice packaging with respect to the addition of sugar, meaning declarations such as "100% natural" would no longer be valid.

This application note is a summary of the work by Psomiadis et al (2018)[1] and focusses on the carbon isotope fingerprint data from pulp and sugar derived from coconuts and commercially available coconut water. For full details presented by the authors, the interested reader is directed to their publication[1].

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Isotope fingerprints of coconut juice

The carbon isotope fingerprint ($\delta^{13}C$ values) of plants are different because photosynthetic processes and broadly grouped as C3, C4 and CAM plant types. Consequently, the $\delta^{13}C$ values of coconut juice is unique and distinguishable from sugar derived from sugar cane, for example. Coconut juice is extracted from the center of a coconut, which grow on coconut trees (*Cocos nucifera*) and are part of the C3 plant family. Sugar derived from sugar cane (*Saccharum spp.*) are part of the C4 plant family. It is known that C3 plants have a carbon isotope fingerprint between -33‰ to -22‰[2,3] and C4 plants have a carbon isotope fingerprint between -16‰ to -8‰[2,3], providing a framework to differentiate.

Sample handling and analytical setup

In this study, the carbon isotope fingerprints of (i) 30 authentic pulp, total sugars and glucose, fructose and sucrose were characterized based on extractions from coconuts in the laboratory and (ii) pulp, total sugars and glucose, fructose and sucrose from 24 commercially available coconut waters (bottled). All samples were treated according to the ENV12140 for isotope analysis of sugar addition to juices. For pulp $\delta^{13}C$ measurements by Elemental Analysis Isotope Ratio Mass Spectrometry (EA-IRMS), ca 45 mL of coconut water was centrifuged, the pulp was washed three times with water and then acetone to remove remnant sugars, organic acids and lipids before being dried under vacuum. For total sugar analysis, approximately 2 g of $Ca(OH)_2$ was added to the supernatant and heated in a bath at 90 ˚C for 3 mins, centrifuged and transferred to a beaker and brought to pH 5 using H_2SO_4. For the individual sugars (sucrose, glucose, fructose), about 5 mL of coconut water were centrifuged to remove particles, then filtered through a 0.2 µm filter and stored until analysis by HPLC/EA-IRMS. Samples were bracketed by two internally calibrated acetanilide and sorghum flour secondary standards, which were calibrated to primary standards relative to Vienna Pee Dee Belemnite (VPDB) using IAEA-CH-6 and IAEA-600.

Isotope fingerprints of bulk coconut juice

Carbon isotope fingerprints of the pulp and sugar extracted from authentic coconut waters were characteristic of C3-plants (Figure 1a), in agreement with the range suggested by AIJN 6.27 Reference Guideline[4]. A theoretical cut-off limit of -21‰ is used to determine if the samples measured are of a C3 or C4 plant nature. Using this limit, the bulk measurement of coconut juice pulp and sugar was able to identify 5 of the 24 commercially bought coconut juices as adulterated (21%).

Detection of added sugar to commercial coconut juice

The addition of C4-plant sugar to coconut juice was better detected using the carbon isotope fingerprints of individual sugars (sucrose, glucose and fructose) by comparison with the isotope fingerprint of total sugar (Figure 1b). For the authentic coconut juices, the carbon isotope fingerprints of sucrose, glucose and fructose fell within the expected range[4]. However, when applied to the commercially purchased coconut juices, the approach resulted in enhanced detection of C4-sugar addition by identifying 38% of adulterated samples. Moreover, the limit of detection was improved such that sugar additions of less than 10% can be detected.

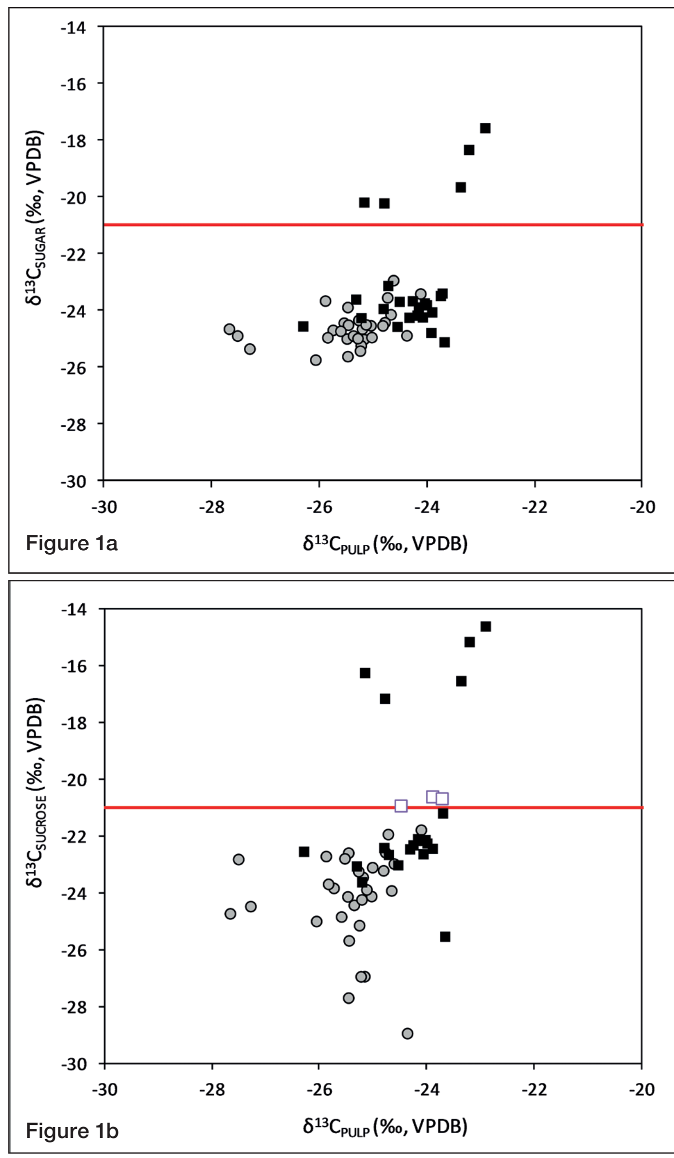

Figure 1. Carbon isotope composition of (a) pulp and total sugars and (b) pulp and sucrose in authentic coconut waters (grey circles) and in commercial bottled coconut waters (squares). The horizontal solid red line indicates the upper limit for C3-plants (- 21% VPDB). The blank (white) squares represent the samples identified as adulterated only by the sucrose carbon isotope analysis (and not by the total sugars).

Summary

The detection of coconut juice adulteration by the addition of C4-plant sugar can be significantly improved by using the carbon isotope fingerprint of specific sugars. It was demonstrated here that 9 out of the 24 tested samples (38%) taken from commercially available outlets, were adulterated.

This study has shown the powerful contribution that carbon isotope fingerprints of sugars in coconut water make to identifying adulterated commercial products. These findings are based on measurements of carbon isotopes using EA-IRMS, such as the Thermo Scientific™ EA IsoLink™ IRMS System and/or Thermo Scientific™ LC IsoLink™ IRMS System (neither system used in this study).

References

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This application report is a summary of the work published by "Psomiadis, D., Zisi, N., Koger, C., Horvath, B., Bodiselitsch, B. (2018). J Food Sci Technol. 55: 2994". No new data or data interpretations have been added to this application note by the author.

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