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Use of stable isotopes of carbon to detect coconut water adulteration

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ABSTRACT: Industrialized coconut water may have been adulterated by adding sugar above the limit permitted by law. According to Brazilian law, industrialized coconut water can receive the maximum addition of 1 g of sugar per 100 mL of the drink. This work aimed to detect adulteration in industrialized coconut water produced in Brazil and measure the relative isotopic enrichment in fresh coconut water, using the techniques of stable isotopes of carbon. Fresh coconut water samples from 13 locations, industrialized coconut water samples of 17 different brands and cane sugar were analyzed by Isotope Ratio Mass Spectrometer coupled to an elemental analyzer. The relative isotopic enrichment found for fresh coconut water samples ranged from -26.40 to -23.76. From 17 brands of coconut water analyzed, 11 were adulterated by excess sugar and two were already adulterated by presenting soluble solids content higher above the threshold permitted by law. In 65 % of Brazilian industrialized coconut water, the amount of exogenous sugar is higher than the limit permitted by law. Most Brazilian companies do not respect the legal limit of adding cane sugar established by law.

Keywords: *Cocus nucifera* L., beverage, isotope analysis, law, fraud

Introduction

The Brazilian law allows for coconut water the addition of the limit of 1 g of sugar per 100 mL of industrialized coconut water to sweeten the final product and establishes a range for soluble solids content (°Brix) from 4.5 to 6.7 (MAPA, 2009a).

Prices of raw materials (juices, concentrates) show seasonal differences, in some cases especially materials with certain properties, e.g., sweet or acidic products. This justifies the addition of industrially produced components, e.g., sugars or acids.

The partial substitution of orange juice concentrate by much cheaper liquid sugars (beet or cane medium invert sugar or mixtures of both) or the preparation of a single strong juice from concentrate (without proper labeling) could result in a remarkable cost advantage (Rossmann, 2001).

Isotope ratio mass spectrometry (IRMS) is a promising tool to investigate food origin; thus, $^{13}C/^{12}C$, $^{18}O/^{16}O$ and $^{2}H/^{1}H$ measurements are highly used in forensic studies to prove product authenticity. This application has been particularly useful in food quality control, as it allows detecting sugar and water added in fruit juices and tracing the geographical origin of food (Magdas and Puscas, 2011).

Plants can be classified according to their photosynthetic cycle C_3 and C_4. Photosynthetic cycle C_3 plants form 3-carbon acid in the capture of atmospheric CO_2 for photosynthesis, reducing CO_2 for phosphoglycerate with the aid of RuBP carboxylase enzyme. This enzyme discriminates $^{13}CO_2$, resulting in isotopic enrichment values ($\delta^{13}C$) relatively low (average -27 ‰ (per thousand)). Photosynthetic cycle C_4 plants form 4- carbon acid in the reduction of CO_2 to aspartic acid or malic acid with the aid of the PEP carboxylase enzyme. Thus, C_4 plants have $\delta^{13}C$ relatively high values (average -13 ‰) (Voznesenskaya et al., 2010).

This difference of photosynthetic cycles makes the isotopic value of derivatives similar to those of the source material (Jahren et al., 2006). The use of IRMS technique has proven very effective in detecting drink adulteration, because it allows identifying the botanical origin of carbon and measuring the amount of each source (C_3 and C_4) in beverages (Magdas et al., 2012).

This study aimed to detect adulteration in the Brazilian industrialized coconut water by adding cane sugar above the legal limit, using the stable isotopes technique.

Materials and Methods

Isotope analyses

All samples and raw materials were analyzed by Isotope Ratio Mass Spectrometer (IRMS) coupled to an elementary analyzer, using tin capsules.

Isotopic enrichment of carbon

The value of relative isotopic enrichment of carbon ($\delta^{13}C$) was calculated by the ratio $^{13}C/^{12}C$ of sample related to the international standard V-PDB (Vienna-PDB). The $\delta^{13}C$ of the sample is mathematically defined by equation 1. The value of $\delta^{13}C$ of coconut water samples were determined by IRMS.

$$\delta^{13}C\ (Sample,\ V-PDB) = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) \quad (1)$$

where: $\delta^{13}C$ = relative isotopic enrichment of carbon-13 of the sample related to international V-PDB standard - dimensionless; R_{sample} = isotopic ratio of sample – dimensionless; $R_{standard}$ = isotopic ratio of V-PDB standard – dimensionless.

Isotope analysis of fresh coconut water

Coconuts from 13 different locations in Brazil were acquired, two fruits from each location. The coconut water was extracted by pedicle ("coconut eye"), bottled in plastic containers and frozen from -15 to -18 °C.

Isotope analysis of cane sugar

Three types of cane sugar were analyzed in this research: crystal, refined and extra fine. Brazilian food industries normally use these types of cane sugar for their low price and availability in the market. Crystal sugar was ground in a cryogenic mill with liquid nitrogen during 3 min to obtain a homogeneous material with fine texture (≤ 65 µm).

Development of legality curve

The Brazilian law (MAPA, 2009b) establishes that coconut water may receive the maximum amount of sugar of 1 g 100 mL^{-1}. This information allowed to calculate the percentage of C_3 carbon source depending on soluble solid content of beverages, according to equations 2 and 3. The legality curve is represented in a Cartesian graph (Figure 1), where soluble solids contents are located in axis x and percentage of theoretical values of C_3 carbon source in axis y.

The percentage of theoretical C_3 carbon was calculated based on beverages soluble solids content, by equations 2 and 3 (Figueira, 2013).

$$B_{fresch\ coconut\ water} * M_{fresh\ coconut\ water} + B_{Cane\ sugar} \times M_{Cane\ sugar} = B_{Industrialized\ coconut\ water} \times M_{Industrialized\ coconut\ water} \quad (2)$$

$$\%C_3 = \frac{B_{fresh\ coconut\ water} * M_{fresh\ coconut\ water}}{B_{fresh\ coconut\ water} * M_{fresh\ coconut\ water} + B_{Cane\ sugar} \times M_{Cane\ sugar}} \quad (3)$$

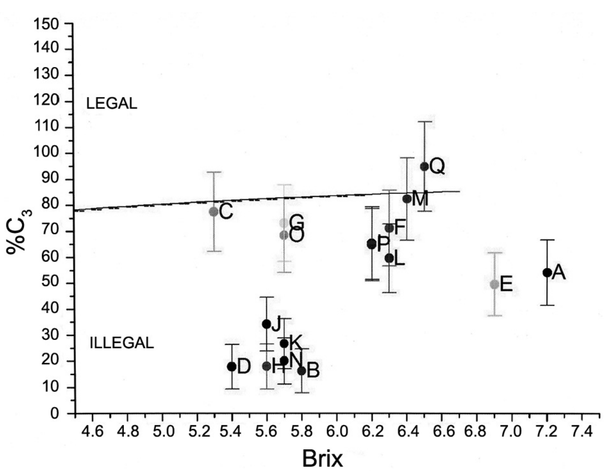

Figure 1 – Legality curve for industrialized coconut water - the theoretical minimum percentage allowed by Brazilian law is shown by the line.

where: B = °Brix; M = mass (g); $\%C_3$ = percentage of carbon from photosynthetic cycle C_3 based on beverage soluble solids content.

Equations 2 and 3 are composed by parameters in grams (g) dimension. The dimension milliliter (mL) was transformed to grams using the corresponding density of coconut water Brix, considering the limit of 1 g 100 mL^{-1} established by the Brazilian law.

Isotope analysis of industrialized coconut water

Coconut waters from 17 different brands in Brazil were analyzed in this work. Three samples of each brand was acquired, with the same volume and manufacturing batch. Two samples of each brand were used for isotope analysis and the third sample was kept frozen as rebuttal evidence. The packages were opened and the beverage was bottled in plastic containers and frozen from –15 to –18 °C.

Determination of adulteration in industrialized coconut water

The percentage of carbon C_3 source was calculated by equations 4 and 5, using the values of $\delta^{13}C$ from industrialized coconut water.

Since the final product (industrialized coconut water) is composed by two isotopically different sources (fresh coconut water – C_3 source and sugarcane – C_4 source), stable isotopes of carbon were used (^{13}C) to quantify the contribution of each source in the final product.

Both raw materials (coconut water and sugar) show natural variation in the value of isotopic enrichment, due to the geographical location where the plant was harvested. Thus, as the origin of raw materials used by the coconut water industry is unknown, natural variations of isotopic enrichment in the country must be considered.

The use of maximum and minimum values of isotopic enrichment found in samples of fresh coconut water (Table 1) and cane sugar (Table 2) allowed to calculate the possible maximum and minimum limits for the industrialized sample, using equations 4 and 5.

$$\delta a * A + \delta b * B = \delta p \quad (4)$$

$$A + B = 1 \quad (5)$$

where: δa = relative isotopic enrichment of carbon C_3 source (fresh coconut water) – ‰; δb = relative isotopic enrichment of carbon C_4 source (cane sugar) – ‰; δp = relative isotopic enrichment of product (sweetened coconut water) – ‰; A = proportion of C_3 source in the product – dimensionless; B = proportion of C_4 source in the product – dimensionless.

This calculated range of maximum and minimum limits is represented in Figure 1 as bars and the midpoint is the mean between these two limits. Therefore, the results are not only a single point, but also a range

Table 1 – Average and standard deviation of relative isotopic enrichment of carbon of fresh coconut water samples according to harvest location.

Location (Coordinates)	$\delta^{13}C$ *10³ (‰)
Bady Bassit – SP (20°55'05" S 49°26'43" W - 510 m)	-23.76 ± 0.04 c
Marília – SP (22°12'50" S 49°56'45" W - 679 m)	-23.82 ± 0.06 c
Botucatu – SP (22°53'09" S 48°26'42" W - 840 m)	-24.47 ± 0.05 bc
São G. da Palha – ES (19°01'01" S 40°32'09" W - 200 m)	-25.69 ± 0.07 bc
Linhares – ES (19°23'28" S 40°04'20" W - 33 m)	-25.47 ± 0.10 bc
Nova Venécia – ES (18°42'38" S 40°24'02" W - 65 m)	-25.56 ± 0.02 bc
Santa M. Eterna – BA (15°51'47" S 39°48" W - 122 m)	-26.11 ± 0.16 bc
Juazeiro – BA (09°24' 50" S 40°30' 10" W - 1100 m)	-26.22 ± 0.03 bc
Rodelas – BA (08°51' 03" S 38°45' 21" W - 309 m)	-25.49 ± 0.07 bc
Aracaju – SE (10°54' 36" S 37°04'12" W - 4 m)	-24.70 ± 0.19 bc
Neópolis – SE (10°19'12" S 36°34'46" W - 30 m)	-26.40 ± 0.12 ab
Maceió –AL (09°39'57" S 35°44'06" W - 7 m)	-26.18 ± 0.05 bc
Sousa – PB (06°45'39" S 38°13'51" W - 220 m)	-23.89 ± 0.14 c

$\delta^{13}C$ = Relative isotopic enrichment of carbon of samples (‰); Means followed by the same letters in the row do not differ by the Tukey test ($p < 0.05$).

Table 2 – High and low values of relative isotopic enrichment of carbon of sugar samples.

Type	Number of brands	Average ± standard deviation $\delta^{13}C$ *10³	High/Low $\delta^{13}C$ *10³
Extra fine	2	-14.16 ± 0.05	-14.28/-14.03
Refined	3	-14.24 ± 0.16	-14.48/-13.84
Crystal	9	-14.21 ± 0.11	-14.79/-13.82
Overall Average	-	-14.20 ± 0.11	-

$\delta^{13}C$ = Relative isotopic enrichment of carbon of sugar (‰).

of possibilities that consider all natural variations of the sources. Both legality curve and range (Figure 1) present values of %C_3. Beverages were considered legal, when their values (midpoints) were above the legality curve. Beverages were considered adulterated, when their values were below the legality curve. Coconut water brands were considered legal, when their midpoints were below the legality curve, but with bars touching it.

Statistical analysis

The data in this research were submitted to analysis of variance and means were compared by the Tukey test and considered significant when $p < 0.05$.

Results and Discussion

Soluble solids analysis

Brands A and E had soluble solids content higher than the threshold established by law (4.5 to 6.7 °Brix), indicating non-compliance with the Brazilian law in this parameter. The remaining samples showed with soluble solid values within the range established by law.

Isotope analysis of fresh coconut water and sugar

Fresh coconut water

The relative isotopic enrichment values of fresh coconut water are shown in Table 1. Despite the geographical distance between São Paulo and Paraíba States, $\delta^{13}C$ values do not differ, probably due to several conditions where the coconut was harvest (soil, climate, water). Although the results in this research indicate no relationship between ^{13}C values and latitude of harvesting, this subject needs to be further analyzed to create a Brazilian database for $\delta^{13}C$ of coconut water and investigate the reasons for such variations.

Sugar

The data presented in Table 2 shows variations in $\delta^{13}C$ values of commercial sugar analyzed in this work. Higher variation was found in crystal sugar (from -14.79 ‰ to -13.82 ‰), probably because the number of samples was higher for this type of sugar.

Other authors have published isotope values of cane sugar (refined and crystal). Banerjee et al. (2015) and Figueira et al. (2013) found values from -12.50 ‰ to -11.90 ‰ and -13.06 ‰ to -12.51 ‰, respectively, and Jahren et al. (2006), from -12.71 ‰ to -11.59 ‰. Due to its influence on results, this high variation (3 ‰) must be considered in the calculation of results.

Development of legality curve

Table 3 presents the data used to calculate the theoretical values of %C_3 for legality curve. Each value of the right column (%C_3) indicates the legal limit according to their Brix value of the theoretical drink sweetened using 1 g of cane sugar per amount of final product in mass (corresponding to 100 mL).

The legality curve was set up from 4.5 to 6.7 °Brix, in compliance with the law. If sample %C_3 is lower than 4.5 °Brix or higher than 6.7 °Brix, it is already adulterated. No sample was found below 4.5 °Brix in this research.

Table 3 – Calculation of percentage of theoretical C_3 source of industrialized coconut water.

Brix of fresh coconut water	Cane sugar*	Brix of industrialized coconut water**	$\%C_3$ **
3.6	1	4.5	78
3.7	1	4.6	79
3.8	1	4.7	79
3.9	1	4.8	79
4.0	1	4.9	80
4.1	1	5.0	80
4.2	1	5.1	81
4.3	1	5.2	81
4.4	1	5.3	81
4.5	1	5.4	82
4.6	1	5.5	82
4.7	1	5.6	82
4.8	1	5.7	83
4.9	1	5.8	83
5.0	1	5.9	83
5.1	1	6.0	84
5.2	1	6.1	84
5.3	1	6.2	84
5.4	1	6.3	84
5.5	1	6.4	85
5.6	1	6.5	85
5.7	1	6.6	85
5.8	1	6.7	85

*(g/corresponding mass (g) from multiplying 100 mL by each density value); **theoretical.

Determination of adulteration in industrialized coconut water

The relative isotopic enrichment values and concentration ranges of C_3 source of industrialized coconut water are shown in Table 4.

The midpoints of these ranges were inserted into the graph faced with the legality curve (Figure 1) to determine the legality of industrialized beverages.

Column 1 of Table 4 shows the means of isotopic enrichment found in industrialized coconut water samples and column 2 displays higher and lower percentage values of the C_3 source range. Column 3 shows the midpoint between the two values of column 2.

These figures show that brands B, D, H, I, J, K, L, N, P are in the "illegal" region determined by the curve. Brands C, F, G, M, O and Q, whose range touches the legality curve, were considered legal. Because I and P samples have a similar values of °Brix and C_3 percentages, they are superimposed on the graph, which blocks the view.

The amount of coconut water adulterated with sugar excess was approximately 65 %, considered high. It is recommended that Ministry of Agriculture take actions with companies that commit adulteration, intentionally or not, on their beverages to ensure better quality of products, providing obvious benefits to costumers.

Table 4 – Average and standard deviation of soluble solids contents, relative isotopic carbon enrichment of the samples, range of C_3 source percentage contribution in industrialized coconut water, and midpoint of this range (n = 2).

Sample	Soluble solids (°Brix)	1	2	3
A	7.2 ± 0.00	-20.06 ± 0.07	67-41	54
B	5.8 ± 0.00	-16.00 ± 0.05	24-8	16
C	5.3 ± 0.00	-22.56 ± 0.20	93-62	77
D	5.5 ± 0.06	-16.17 ± 0.28	26-9	18
E	6.9 ± 0.05	-19.57 ± 0.08	62-37	49
F	5.8 ± 0.06	-21.89 ± 0.01	86-57	71
G	5.7 ± 0.00	-22.09 ± 0.07	88-58	73
H	5.6 ± 0.00	-16.19 ± 0.56	26-9	18
I	6.3 ± 0.06	-21.26 ± 0.09	79-51	65
J	5.6 ± 0.00	-17.92 ± 0.11	44-24	34
K	5.7 ± 0.00	-17.12 ± 0.09	36-17	27
L	6.3 ± 0.00	-20.64 ± 0.39	73-46	59
M	6.4 ± 0.00	-23.09 ± 0.02	98-66	82
N	5.7 ± 0.00	-16.41 ± 0.15	29-11	20
O	5.7 ± 0.00	-21.59 ± 0.14	83-54	68
P	6.2 ± 0.00	-21.20 ± 0.36	79-51	65
Q	6.5 ± 0.00	-24.43 ± 0.53	112-78	95

1 = $\delta^{13}C*10^3$ (‰) ± Standard deviation; 2 = C_3 source concentration range (%); 3 = Midpoint of C_3 source concentration range (%).

Conclusions

The Brazilian companies of bottled coconut water use cane sugar for correction and standardization of drinks soluble solids content.

Samples A and E are adulterated because of the high content of soluble solids (7.2 °Brix and 6.9 °Brix, respectively) that exceed the upper limit of 6.7 °Brix required by law.

Eleven of the 17 analyzed brands are adulterated by excess sugar.

Most (65 %) Brazilian companies do not respect the legal threshold of adding cane sugar 1 g 100 mL^{-1}; nevertheless, some companies (35 %) add sugar in compliance with current legislation.

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Authors' Contributions

Conceptualization: Imaizumi, V.M., Ducatti, C., Venturini-filho, W.G. Data acquisition: Imaizumi, V.M., Ducatti, C., Venturini-filho, W.G. Data analysis: Sartori, M.M.P. Design of methodology: Ducatti, C., Venturini-filho, W.G. Writing and editing: Imaizumi, V.M., Ducatti, C., Venturini-filho, W.G., Sartori, M.M.P.

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