ISSN 2359-6562 (ONLINE) 2359-6562 (CD-ROM)

ISOTOPIC COMPOSITION ($\delta^{13}C$) AND AUTHENTICITY OF COMERCIAL PULP, TROPICAL JUICES AND NECTARS OF MANGO

RICARDO FIGUEIRA[1], VITOR MASSAMI IMAIZUMI[1], THALIA LEE LOPES DE ANDRADE[1], WALDEMAR GASTONI VENTURINI FILHO[1]

[1]Departamento de Produção Vegetal/Área Horticultura, Faculdade de Ciências Agronômicas, UNESP. Av. Universitária, 3780 - Altos do Paraíso, CEP 18610-034, Botucatu, SP, Brasil. ricardo.figueira@unesp.br; vtr_massami@hotmail.com; thalialda@hotmail.com; waldemar.venturini@unesp.br

ABSTRACT: The aim of this study was to quantify the carbon-13 of C_3 photosynthetic cycle in commercial pulps, tropical juices and nectars of mango through the technique of stable isotopes of carbon to identify the beverages at odds with Brazilian law. The relative isotope enrichment of cane sugar, additives, laboratory-fabricated beverages and commercial beverages was measured in Isotopic Ratios Mass Spectrometer (IRMS). To estimate the error of the isotopic methodology sweetened tropical juices and nectars were produced in laboratory according to their Identity and Quality Standard (IQS) and also adulterated beverages with amount of pulp below of the established by Brazilian law. In these beverages, the theoretical percentage of C_3 source was calculated and the practical percentage was determined in IRMS. The difference between these measurements represented the error of the method. Twenty-two brands of non-alcoholic mango beverages were analyzed. According to the minimum soluble solids contents established by Brazilian legislation, two brands were classified as adulterated. However, isotopic analysis of carbon indicated that 12 commercial beverages were adulterated. Even in a small number of analyzed samples, adulterated beverages (54.55 %) were found. This fact demonstrates the need for greater control in this agribusiness sector.

Keywords: *Mangifera indica*, legislation, quality, adulteration, carbon-13

COMPOSIÇÃO ISOTÓPICA ($\delta^{13}C$) E AUTENTICIDADE DE POLPAS, SUCOS TROPICAIS E NÉCTARES COMERCIAIS DE MANGA

RESUMO: O objetivo deste trabalho foi quantificar o carbono do ciclo fotossintético C_3 em polpas, sucos tropicais e néctares de manga comerciais por meio da técnica dos isótopos estáveis do carbono para identificar as bebidas em desacordo com a legislação brasileira. O enriquecimento isotópico relativo dos açúcares de cana, aditivos, bebidas fabricadas em laboratório e bebidas comerciais foi mensurado no Espectrômetro de Massa de Razões Isotópicas (IRMS). Para estimar o erro do método isotópico foram produzidos em laboratório sucos tropicais adoçados e néctares conforme a legislação brasileira e também bebidas adulteradas (com quantidade insuficiente de polpa). Nestas bebidas foi calculada a porcentagem teórica de fonte C_3. A porcentagem prática foi determinada no IRMS. A diferença entre essas quantificações representou o erro do método. Para determinar a legalidade das bebidas comerciais foi calculado o Limite de Legalidade tendo como referência o PIQ de cada bebida. Vinte e duas marcas de bebidas não alcoólicas de manga foram analisadas. De acordo com o teor de sólidos solúveis estabelecido pela legislação, duas marcas foram classificadas como adulteradas. Porém, a análise isotópica do carbono quantificou a adulteração em doze marcas. Mesmo com um pequeno número de amostras analisadas, 54,55 % estavam adulteradas. Esse fato demonstra a necessidade de maior controle nesse setor do agronegócio.

Palavras-chave: *Mangifera indica*, legislação, qualidade, adulteração, carbono-13.

Recebido em 11/11/2021 e aprovado para publicação em 20/12/2021
DOI: http://dx.doi.org/10.17224/EnergAgric.2022v37n1p39-51

1 INTRODUCTION

Juice is a beverage that contains 100 % of fruit juice in its composition, except for very viscous fruits (i.e. mango fruit), which require dilution of its juice or pulp. When such dilution needs to be performed in a juice obtained from a tropical fruit, the beverage is called tropical juice. Tropical juice can be sweetened with sugar. This beverage must be called tropical juice followed by the name of the fruit and the designation “sweetened”. The mango pulp content used in the tropical juice production should be higher than required for nectar (BRASIL, 2009).

According to the Identity and Quality Standard (IQS) tropical fruit juice must be produced with at least 60 % pulp (m/m) and soluble solids content equal to or greater than 10 ºBrix. The sweetened version must contain at least 50 % pulp (m/m) and soluble solids content greater than or equal to 11 °Brix. Mango nectar must be prepared with a minimum of 40 % pulp (m/m) and 10 ºBrix (BRASIL, 2003). The mango pulp must present at least 11 °Brix (BRASIL, 2000).

In order to reduce production costs, the main problem of authenticity in sweetened beverages, such as sweetened tropical juices and nectars, is the use of mango pulp below the minimum value of soluble solids content required by law. The adulteration occurs when sugar cane is added in beverages that this addition is not allowed (pulps, not-sweetened tropical juices and low calorie nectars). The technique of stable isotopes of carbon has been used in official surveillance institutions as an instrument for the assessment of fraudulent products (BRASIL, 2001; BRASIL, 2007a).

Plants generally have an isotopic carbon signature that varies according to the fixation mode of atmospheric CO_2 during their photosynthetic cycles. The two main biochemical pathways for carbon fixation are from C_3 and C_4 plants. All species of C_3 plants (mango, guava, peach, etc.) reduce CO_2 to 2 molecules of acid 3-phosphoglycerate (3 carbon atoms) resulting in $\delta^{13}C$ values around -28 ‰. All C_4 species (sugar cane, corn and sorghum) reduce CO_2 to aspartic acid or malic acid (both with 4 carbon atoms) showing mean values of $\delta^{13}C$ of -13 ‰. This difference between C_3 and C_4 plants is also found in their products and derivatives (ROSSMANN, 2001; OLIVEIRA *et al*., 2002).

Most isotopic techniques require the use of an isotopic database of raw materials (juice or fruit pulp and cane sugar) as a reference for comparison, in order to estimate the composition of the products to be analyzed. In some cases, the database can be replaced by the isotopic analysis of an internal standard. The use of an internal component as an isotopic reference reduces quantification errors due to the isotopic variability of the raw materials. For mango products, insoluble solids may be used as an internal standard. However, cane sugar does not have an internal reference, requiring the use of isotopic values from a database of different types of sugars (KELLY, 2003).

The aim of this work was to determine the isotopic composition of mango pulps, tropical juices and nectars, quantifying the percentage of carbon-13 from photosynthetic cycle C_3 plant, in order to identify the products that were not in accordance with Brazilian legislation.

2 MATERIAL AND METHODS

The isotopic dilution method is based on the mixture of two sources isotopically different, creating a product whose composition reflects the isotopic contribution of these two sources, as well as the relative proportion of each one of them (OLIVEIRA *et al*., 2002). When mixing mango pulp (C_3) and cane sugar (C_4) for the production of sweetened tropical juice and/or nectar, the beverage will have an intermediate isotopic value between the two sources, depending on the proportion of each. The C_3 and C_4 sources quantification can be obtained by Equations 1 and 2:

$$\delta a * A + \delta b * B = \delta p \quad (1)$$

$$A + B = 1 \quad (2)$$

The symbols of equations 1 and 2 represent: δa, δb and δp = relative isotopic enrichment of the carbon sources C_3, C_4 and the product, respectively (dimensionless); A and B

= relative proportion of the sources C_3 and C_4 in the product, respectively (dimensionless). Therefore, δa represents the isotopic value of insoluble solids (internal-standard), δb represents the isotopic value of cane sugar and δp is the isotopic value of the beverage.

Isotopic analyzes were performed on cane sugar, additives (citric acid, ascorbic acid, synthetic mango flavor, acesulfame potassium, sodium benzoate and sucralose), laboratory-made beverages (sweetened tropical juice and nectar) and commercial beverages (pulp, sweetened and unsweetened tropical juice, conventional and low calorie nectar).

Three samples of each commercial brand from the same batch were bought in supermarkets located in São Paulo state. From 18 brands of mango beverages acquired, there were two of pulp, three of sweetened tropical juice, four of unsweetened tropical juice, six of nectar and three of low calorie nectar. The samples were preparing to isotopic analysis according to Rossmann *et al*. (1997). The relative isotopic enrichment was measured in the Isotopic Ratio Mass Spectrometer (IRMS) (Delta S Finnigan Mat).

2.1 Method error

In order to estimate the error of the isotopic method, sweetened tropical juices and nectars were made in the laboratory according to each IQS. Adulterated beverages were also produced with a quantity of pulp below that established by IQS (in duplicate).

The sweetened tropical juices were fabricated with 11 °Brix (minimum value allowed - BRASIL, 2003) and with the addition of 10, 15, 20 to 60 % (m/m) of pulp. The nectars were produced with 10 ºBrix (minimum value allowed - BRASIL, 2003) and with the addition of 10, 15, 20 to 60 % (m/m) of pulp. These beverages were made using mango pulp at 11 ºBrix (minimum soluble solids content of mango pulp - BRASIL, 2000).

The beverages were prepared and analyzed as previously described. With the results of isotopic analysis, it was possible to calculate the percentage of C_3 source in a practical way (Equations 1 and 2).

From the laboratory production data of sweetened tropical juices and nectars, it was also possible to measure the theoretical percentage of C_3 source (Equations 3 and 4).

$$°Brix_{Pulp}*M_{Pulp} + °Brix_{Sugar}*M_{Sugar} + °Brix_{Water}*M_{Water} = °Brix_{Beverage}*M_{Beverage} \qquad (3)$$

$$\% C_3 = \frac{°Brix_{Pulp} * M_{Pulp}}{°Brix_{Pulp} * M_{Pulp} + °Brix_{Sugar} * M_{Sugar}} * 100 \qquad (4)$$

The symbols of Equations 3 and 4 represent: Brix = soluble solids content of mango pulp (11 °Brix), cane sugar (100 °Brix), water (0 °Brix) and beverage (sweetened tropical juice: 11 ºBrix; nectar: 10 ºBrix); M = mass of the pulp, sugar, water and beverage.

The theoretical percentage was compared with the practical percentage of C_3 source. The difference between these quantifications, in module, represented the error of the method.

2.2 C_3 source percentage comparison between mango beverages produced with and without additives

In order to evaluate the influence of the additives in the quantification of C_3 source, beverages with addition of additives and beverages without additives were produced.

The unsweetened tropical juices were produced using 60 % (m/m) of pulp at 11 °Brix (BRASIL, 2000; BRASIL, 2003). The low calorie nectars were prepared with 40 % (m/m) of pulp at 11 °Brix (BRASIL, 2000; BRASIL, 2003). In both beverages, there was no addition of sugar cane (BRASIL, 2003; BRASIL, 2009).

The additives used in the production of sweetened tropical juices and conventional nectars, as well as their respective amounts were: ascorbic acid (0.05 g.100 mL^{-1}), citric acid (0.3 g.100 mL^{-1}) and synthetic mango flavor (0.03 g.100 mL^{-1}). For tropical unsweetened juices were: citric acid (0.3 g.100 mL^{-1}) and sodium benzoate (0.05 g.100 mL^{-1}). For low-calorie nectars, the same additives used in conventional nectars were added, in addition to acesulfame potassium (0.04 g.100 mL^{-1}) and sucralose (0.03 g.100 mL^{-1}). These quantities were reported by Brazilian fruit beverage industry, except sodium benzoate that is determined by Brasil (2007b).

After the isotopic analysis of the beverages produced with and without additives, the percentages of C_3 source (Equations 1 and 2) were calculated. The values obtained were compared by statistical tests.

2.3 Legality limit development

The legality limit (LL) provides the minimum concentration of C_3 source that a beverage must contain to be considered legal, under Brazilian law. The legality limit is a theoretical measure of the amount of C_3 source and uses the specific IQS of each beverage as reference.

Beverages ranging from 11 to 15 °Brix (with 0.5 °Brix increase) produced with 50 % (m/m) of pulp were adopted as reference to calculate the legality limit of sweetened tropical juices. For nectars, beverages ranging from 10 to 14 °Brix (with 0.5 °Brix increase) produced with 40 % pulp (m / m) were adopted as reference to calculate the legality limit. These beverages were produced with mango pulp standardized for 11 ºBrix (BRASIL, 2000; BRASIL, 2003).

From these data and using Equations 3 and 4 the C_3 source minimum percentage for each drink was calculated. The obtained values were related to the respective soluble solids contents (°Brix) in a Cartesian graph. The resulting curve generated the legality limit.

For not penalizing the manufacturers of commercial mango beverages, the calculated values for the legality limit of sweetened tropical juices and nectars were subtracted from the error of the method.

Unsweetened tropical juices, low-calorie nectars and pulps are not allowed to contain cane sugar in their composition (BRASIL, 2000; BRASIL, 2003). Theoretically, they should present 100 % of the C_3 source, regardless of the °Brix of the samples. However, this value is rarely obtained. The inaccuracies that occur during the instrumental preparation and analysis of the beverage (method error), including the presence of additives, contribute to reduce the C_3 source quantification. Thus, for beverages that do not contain cane sugar, the percentage of C_3 source measured for beverages produced with additives was adopted as legality limit, according to item 2.2.

2.5 Adulteration determination of mango commercial beverages

In order to calculate the percentage of C_3 source (Equations 1 and 2) of commercial beverages, the isotopic value of the insoluble solids (δa) and the isotopic value of the commercial drink (δp) were used. For beverages with cane sugar in their composition (sweetened tropical juices and nectars) the lightest and heaviest isotopic value of cane sugar (δb - database) was used. For commercial beverages that cane sugar is not allowed in the composition (unsweetened tropical juice, low calorie nectar and pulp) (BRASIL, 2000; BRASIL, 2009), the value of 0.00 % was adopted in B.

The C_3 source percentages of the commercial beverages were related to their own Brix value on a Cartesian graph. In this same graph, the values of the legality limit were inserted, discounting the error of the method. When the C_3 source quantification band was above or overlaid the legality limit, the beverage was considered legal. If the band was below this limit, the beverage was classified as adulterated.

2.6 Statistical analyzes

Statistical analyzes were performed to compare the different types of cane sugar (Tukey's test; α = 0.05) and beverages produced with and without additives (t-test; α = 0.05) using the free software Assistat (2011), version 7.6 beta.

3 RESULTS AND DISCUSSION

3.1 Isotopic analyzes of cane sugar, additives and laboratory made beverages

Published papers on isotope analysis in orange nectars (FIGUEIRA *et al.*, 2011b), cashew juices and pulps (FIGUEIRA *et al.*, 2011c) and peach nectars (NOGUEIRA *et al.*, 2011) showed an average value of cane sugar between -13.11 ‰ at -12.83 ‰, close to that found in the present study (Table 1). These variations may have occurred because environmental (soil irradiation, moisture and salinity, etc.) and biological factors (photosynthetic capacity, genetic variation, competition, etc.) may influence carbon isotopic composition in C_3 and C_4 plants (BOUTTON, 1996). There was no statistical difference between the four types of cane sugar analyzed (Table 1).

The relative isotopic enrichment of the acesulfame potassium (-28.56 ± 0.01 ‰) and the sodium benzoate (-29.91 ± 0.19 ‰) were similar to those of plants with C_3 photosynthetic metabolism, while the values of ascorbic acid (-12.36 ± 0.14 ‰) and citric acid (-12.85 ± 0.09 ‰) were similar to those measured in C_4 plants. The synthetic mango flavor (-21.24 ± 0.01 ‰) and sucralose (-18.49 ± 0.05 ‰) had intermediate isotopic values between the C_3 and C_4 sources. The isotopic values of ascorbic acid and citric acid were similar to those reported by Figueira *et al.* (2011b) (-12.17 ‰ and -13.45 ‰, respectively). The value of sodium benzoate was similar to that found by Figueira *et al.* (2011c)
(-29.46 ‰). The acesulfame potassium and sucralose values supports data from Nogueira *et al.* (2011) (-28.31 ‰ and -18.49 ‰, respectively). No researches were found to compare the isotopic value of synthetic mango flavor.

As expected, the isotopic values of sweetened tropical juices and nectars produced in the laboratory became lighter as the addition of mango pulp was increased. The isotopic values of the insoluble solids (internal standard) did not vary in both products regardless of the amounts of pulp (C_3 source) or cane sugar (C_4 source) added (Table 2). The use of insoluble solids as an internal isotopic reference allows the identification of the isotopic value of the raw material of C_3 origin. This observation had already been verified by Jamin *et al*. (1998) and Kelly (2003), who developed their research analyzing the internal standard of juices without quantifying the sugar used in adulteration.

Table 1. Relative isotopic enrichment ($\delta^{13}C$) of cane sugars. Database for δb.

Sugar	**n**	**Mean ± Standard deviation (lower/higher)**
Crystal	9	-12.93a[1] ± 0.19 (-12.62/-13.17)
Fine	5	-13.29a ± 0.62 (-12.78/-14.09)
Liquid	2	-12.94a ± 0.23 (-12.77/-13.10)
Invert	3	-13.38a ± 0.42 (-13.00/-13.83)
Overall mean ± Standard deviation		-13.10 ± 0.40

[1]Tukey's test (α = 0.05).

Table 2. Relative isotopic enrichment ($\delta^{13}C$) of the insoluble solids fraction (δa) and mango sweetened tropical juices and nectars made in laboratory (δp).

Pulp (%)[1]	δp ($\delta^{13}C$ * 1000)		δa ($\delta^{13}C$ * 1000)	
	Beverages	**Standard deviation**	**Insoluble solids**	**Standard deviation**
		Sweetened Tropical Juice		
10	-14.10	0.09	-27.82	0.01
15	-15.11	0.07	-27.69	0.04
20	-15.48	0.11	-27.98	0.05
25	-16.39	0.03	-27.65	0.03
30	-16.84	0.09	-28.00	0.01
35	-17.77	0.04	-27.56	0.15
40	-18.21	0.01	-27.98	0.04
45	-19.16	0.07	-27.65	0.03
50	-19.57	0.13	-27.98	0.01
55	-20.09	0.14	-27.94	0.03
60	-21.26	0.06	-27.36	0.04
		Nectar		
10	-14.47	0.01	-27.87	0.10
15	-15.21	0.01	-28.26	0.01
20	-15.97	0.01	-27.64	020
25	-16.97	0.20	-28.28	0.08
30	-17.51	0.07	-27.69	0.09
35	-18.19	0.04	-28.40	0.16
40	-19.36	0.13	-28.49	0.06
45	-19.76	0.05	-28.17	0.11
50	-20.85	0.01	-27.81	0.17
55	-21.11	0.03	-28.32	0.10
60	-22.41	0.01	-27.79	0.16

[1]percentage of pulp corrected to 11 ºBrix

3.2 Method error

In order to calculate the practical quantity of the C_3 source of the beverages produced in the laboratory (Table 3), the values of the relative isotopic enrichment of the solids insoluble in δa (Table 2), cane sugar in δb (-12.67 ‰) and laboratory-made beverages in δp (Table 2) were used, in Equations 1 and 2.

The error of the method for sweetened tropical juices was 2.32 ± 1.97 %. For nectars, the error was 1.81 ± 2.01 % (Table 3). Adding the mean of the errors to the standard deviation, the values of 4.29 % for tropical sweetened juices and 3.82 % for nectars was obtained.

Table 3. Comparison between theoretical and practical percentage of the C_3 source of laboratory-made mango sweetened tropical juices and nectar.

Pulp (%)[1]	C_3 theoretical (%)	C_3 practical (%)	|Error|[2] (%)
Sweetened Tropical Juice			
10	10.00	9.44	0.56
15	15.00	16.25	1.25
20	20.00	18.35	1.65
25	25.00	24.83	0.17
30	30.00	27.20	2.80
35	35.00	34.25	0.75
40	40.00	36.19	3.81
45	45.00	43.32	1.68
50	50.00	45.07	4.93
55	55.00	48.59	6.41
60	60.00	58.48	1.52
Mean			2.32
Standard Deviation			1.97
Nectar			
10	10	11.84	1.84
15	15	16.29	1.29
20	20	22.04	2.04
25	25	27.55	2.55
30	30	32.22	2.22
35	35	35.09	0.09
40	40	42.29	2.29
45	45	45.74	0.74
50	50	54.03	4.03
55	55	53.93	1.07
60	60	64.42	4.42
Mean			1.81
Standard Deviation			2.01

[1]percentage of pulp corrected to 11 ºBrix; [2]Module value.

3.3. Comparison of C_3 source measurement between tropical juices and mango nectars produced without and with additives

The quantification of C_3 source in mango sweetened tropical juices, conventional nectars and unsweetened tropical juices produced without additives did not differ statistically from beverages with additives. The relative amount of carbon-13 in the additive was not representative of the amounts of C_3 and C_4 carbons-13 in the raw materials (Table 4).

In the low calorie mango nectars there was a statistical difference between the quantification of the C_3 source of beverages produced with additive compared with beverages produced without additive. The low calorie nectars were produced with two additives with isotopic value similar to C_4 source (ascorbic acid and citric acid) in addition to additives with isotopic values intermediate between the sources C_3 and C_4 (synthetic mango flavor and sucralose), as reported in item 3.1 . In this case, the relative amount of carbon-13 of the additives was significant when compared with the amount of carbon-13 of the raw materials (Tabela 4).

This additive influence in different types of products has been reported in researches of isotopic analysis in pulps, juices and nectars of different flavors: peach (NOGUEIRA *et al*., 2011); orange (FIGUEIRA et al., 2011b); apple (FIGUEIRA *et al.*, 2011a).

Table 4. Statistical comparison between mango beverages produced with and without additives.

Beverage	Additive	C_3 ± Standard deviation (%)
Sweetened tropical juice	-[1]	45.42 ± 1.28 a[3]
	+[2]	46.19 ± 0.59 a
Nectars	-	43.26 ± 0.49 a
	+	42.22 ± 1.56 a
Unsweetened tropical juice	-	97.02 ± 0.47 a
	+	96.47 ± 0.38 a
Low calorie nectars	-	97.19 ± 1.28 a
	+	94.05 ± 1.00 b

[1]Additives abscence; [2]Additives presence; [3]t-test for paired samples (α = 0.05)

3.4. Legality limit development for mango pulps, tropical juices and nectars

For mango sweetened tropical juices and conventional nectars, the legality limit was obtained according to the mass balance shown in Table 5. The values of the legality limit were subtracted from the method error (mean + standard deviation) obtained in item 3.2.

Table 5. Mass balance (theoretical) to obtain the legality limit of mango sweetened tropical juices and nectars.

Pulp			Sugar		Water		Beverage		LL	LL – Error
%	°Brix	g	°Brix	g	°Brix	g	°Brix	g	(%C_3)[1]	(%)[2]
Sweetened tropical juice										
50	11	125	100	13.75	0	111.25	11.0	250	50.00	45.71
50	11	125	100	15.00	0	110.00	11.5	250	47.83	43.54
50	11	125	100	16.25	0	108.75	12.0	250	45.83	41.54
50	11	125	100	17.50	0	107.50	12.5	250	44.00	39.71
50	11	125	100	18.75	0	106.25	13.0	250	42.31	38.02
50	11	125	100	20.00	0	105.00	13.5	250	40.74	36.45
50	11	125	100	21.25	0	103.75	14.0	250	39.29	35.00
50	11	125	100	22.50	0	102.50	14.5	250	37.93	33.64
50	11	125	100	23.75	0	101.25	15.0	250	36.67	32.38
Nectar										
40	11	100	100	14.00	0	136.00	10.0	250	44.00	40.18
40	11	100	100	15.25	0	134.75	10.5	250	41.90	38.08
40	11	100	100	16.50	0	133.50	11.0	250	40.00	36.18
40	11	100	100	17.75	0	132.25	11.5	250	38.26	34.44
40	11	100	100	19.00	0	131.00	12.0	250	36.67	32.85
40	11	100	100	20.25	0	129.75	12.5	250	35.20	31.38
40	11	100	100	21.50	0	128.50	13.0	250	33.85	30.03
40	11	100	100	22.75	0	127.25	13.5	250	32.59	28.77
40	11	100	100	24.00	0	126.00	14.0	250	31.43	27.61

[1]Legality limit; [2]Legality limit subtracted from method error (sweetened tropical juice = 4.29 %; nectar = 3.82 %).

Unsweetened tropical juices and low-calorie nectars cannot contain cane sugar in their compositions (BRASIL, 2003; BRASIL, 2009). Therefore, the quantification of C_3 source in these products should be 100 %, regardless of its ºBrix. However, as verified in item 3.3, the percentage of C_3 source in these drinks was lower than expected. In addition, in low calorie nectars, there was a statistical difference between the quantification of C_3 source of beverages produced with and without additives. Thus, the legality limit for both beverages was defined as the percentage of C_3 source measured for products produced with additives, subtracted from the standard deviation (unsweetened tropical juices: 96.47 % - 0.38 % = 96.09 %, low calorie nectars: 94.05 % - 1.00 % = 93.05 %). The mango pulps were analyzed like unsweetened tropical juices.

3.5. Isotopic analysis and legality determination of mango pulps, tropical juices and nectars

According to IQS and considering that it is not allowed to add sugar in low calorie nectar, the minimum content of soluble solids was calculated in 4.40 °Brix. Taking into account only the soluble solids contents of the mentioned products, it is possible to say that most of mango beverages analyzed in this study were in compliance with Brazilian legislation. Exceptions were samples 09 and 10 (low calorie nectar) which presented soluble solids content lower than expected (Tables 6 and 7).

In order to calculate the percentage of C3 source in commercial beverages (Equations 1 and 2), the isotope value of the insoluble solids in δa and the commercial isotope value in δp (Tables 6 and 7) were used. For beverages containing cane sugar in their composition (sweetened tropical juices and nectars) the lightest (-14.09 ‰) and heavier (-12.62 ‰) isotopic values of cane sugar were used in δb (Database - Table 1). For beverages that do not contain cane sugar (sweetened tropical juice, low calorie nectar and pulp), the value of 0.00 % in B was adopted.

Table 6. Relative isotopic enrichment ($\delta^{13}C$) and percentage of C_3 source of mango commercial sweetened tropical juices and nectars and insoluble solids fraction.

Nº	ºBrix	δa ($\delta^{13}C$ * 1000)		δp ($\delta^{13}C$ * 1000)		C_3 Minimum (%)[1]	C_3 Maximum (%)[2]
		Insoluble solids	Standard deviation	Commercial beverage	Standard deviation		
Sweetened tropical juice							
94	12.8	-29.12	0.12	-17.82	0.10	24.82	31.52
95	11.5	-28.68	0.04	-18.17	0.04	27.96	34.56
98	11.6	-28.60	0.03	-18.82	0.00	32.60	38.80
Nectar							
04	14.5	-29.55	0.20	-19.02	0.19	31.89	37.80
05	11.5	-28.20	0.04	-17.80	0.04	26.29	33.25
06	12.4	-28.46	0.01	-18.65	0.07	31.73	38.07
07	12.3	-28.50	0.08	-18.94	0.04	33.66	39.80
08	12.4	-27.65	0.07	-16.38	0.02	16.89	25.02
93	13.2	-28.28	0.02	-16.76	0.03	18.82	26.44

[1]C_3 source (%) when δb = -14.09 ‰ (Equations 1 and 2); [2]C_3 source (%) when δb = -12.62 ‰ (Equations 1 and 2).

Table 7. Relative isotopic enrichment ($\delta^{13}C$) and percentage of C_3 source of mango unsweetened tropical juices and low calorie nectars and insoluble solids fraction.

Nº	ºBrix	δa ($\delta^{13}C$ * 1000) Insoluble solids	Standard deviation	δp ($\delta^{13}C$ * 1000) Commercial beverage	Standard deviation	C_3 (%)[1]
Unsweetened tropical juice						
11	12.1	-28.54	0.16	-27.25	0.04	95.48
12	11.4	-28.55	0.08	-27.62	0.13	96.74
13	10.0	-27.21	0.13	-23.05	0.10	84.71
97	11.6	-27.55	0.03	-27.81	0.13	100.94
Pulp						
02	14.4	-28.99	0.16	-27.75	0.01	95.72
03	28.0	-28.90	0.08	-27.67	0.09	95.74
60	15.3	-27.78	0.01	-27.06	0.11	97.41
82	14.4	-28.85	0.06	-28.48	0.02	98.72
99	12.3	-28.32	0.05	-27.72	0.02	97.88
100	11.8	-27.65	0.02	-26.45	0.09	95.66
Low calorie nectar						
09	4.2	-28.57	0.12	-26.50	0.04	92.75
10	4.1	-28.31	0.04	-27.97	0.04	98.80
96	4.8	-29.14	0.04	-27.85	0.01	95.57

[1]Percentage of C_3 source when B = 0.00 ‰ (Equations 1 and 2).

No research was found with mango based non-alcoholic beverages that reported the isotopic values of these products.

The samples 4, 6 and 7 (nectars) presented their C_3 source quantification ranges above or overlaid the legality limit and were classified as legal. The samples 94, 95, 98 (sweetened tropical juices) and 5, 8, 93 (nectars) were below the legality limit and were considered as illegal (Figures 1 and 2).

The legality limit for mango unsweetened tropical juice and pulp was estimated at 96.09 % (item 3.4), regardless of the soluble solids content of the product. The samples 12, 97 (unsweetened tropical juice) and 60, 82, 99 (pulp) presented their C_3 source quantification ranges above the legality limit and were classified as legal. The samples 11, 13 (unsweetened tropical juices) and 2, 3, 100 (pulp) were below the legality limit and were considered as illegal (Table 7).

The legality limit for mango low calorie nectar was estimated at 93.05 % (item 3.4), regardless of the soluble solids content of the product. The samples 10 and 96 presented C_3 source percentages above the legality limit and were classified as legal. However, sample 10 was reproved for having soluble solids content below than recommended by Brazilian legislation (4.40 °Brix). Sample 9 had its C_3 source quantification below the legality limit and were in law disagreement.

Figure 1. Legality of commercial sweetened tropical juices.

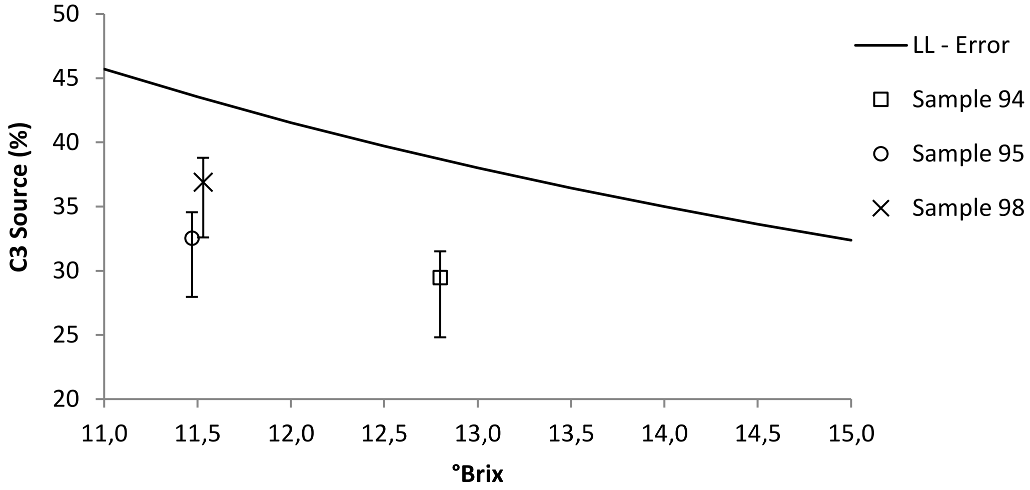
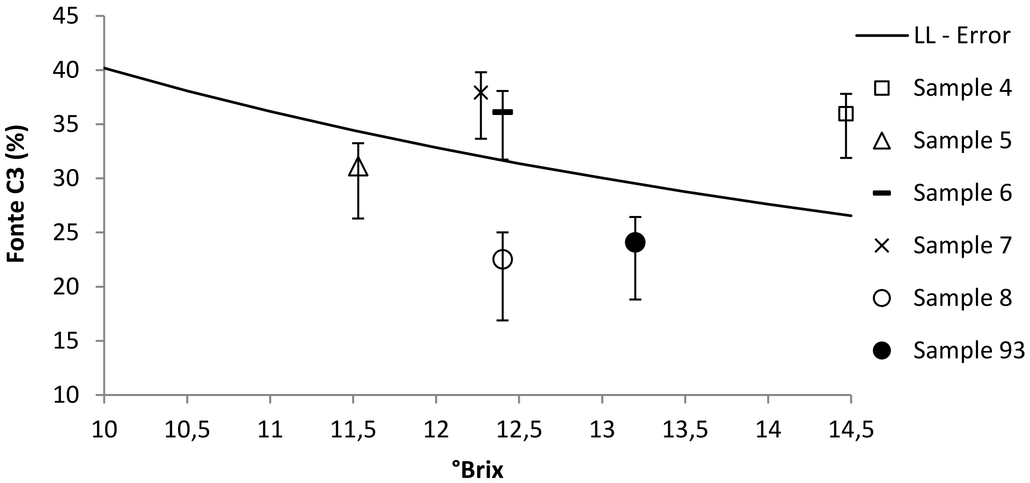


Figure 2. Legality of commercial nectars.



4 CONCLUSIONS

Twenty-two brands of non-alcoholic mango beverages were analyzed. According to the minimum soluble solids contents established by Brazilian legislation, two brands were classified as adulterated. However, isotopic analysis of carbon indicated that 12 commercial beverages were adulterated. This observation confirms the results found in the literature that reports the inefficiency of physical and chemical parameters in the quality control of fruit-based non-alcoholic beverages.

The technique that uses stable isotopes of carbon to verify adulterations in fruit-based non-alcoholic beverages was efficient to measure the amount of C_3 source in commercial mango beverages, allowing a reliable identification of the fraudulent products.

Even in a small number of analyzed samples, adulterated beverages (54.55 %) were found. This fact demonstrates the need for greater control in this agribusiness sector.

Non-alcoholic mango beverages have their own IQS, which has generated specific legality limits for each product. The limit of legality calculated according to the norms established by Brazilian legislation was an important methodological innovation that made the identification of adulteration in beverages.

5 REFERENCES

ASSISTAT. **Assistência estatística.** versão 7.6 beta. Campina Grande: DEAG-CTRN-UFCG, 2011.

BOUTTON, T. W. Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change. *In*: BOUTTON, T. W.; YAMASAKI, S. **Mass spectrometry of soils**. New York: Marcel Dekker, 1996. chap. 2, p. 47-82.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa n. 1, de 07 de janeiro de 2000. Aprova o Regulamento Técnico Geral para fixação dos Padrões de Identidade e Qualidade para Polpa de Fruta. **Diário Oficial da República Federativa do Brasil**: seção 1, Brasília, DF, ano 2000, n. 6, p. 54-58, 10 jan. 2000.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa n. 4, de 05 de fevereiro de 2001. Aprova a Metodologia de Análise da Razão Isotópica $^{13}C/^{12}C$ em Produtos e Subprodutos das Plantas do Ciclo Fotossintético C_3 e C_4. **Diário Oficial da República Federativa do Brasil**: seção 1, Brasília, DF, ano 2001, n. 26, p. 2-4, 8 fev. 2001.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa n. 12, de 04 de setembro de 2003. Aprova o Regulamento Técnico para Fixação dos Padrões de Identidade e Qualidade Gerais para Suco Tropical e Néctar. **Diário Oficial da República Federativa do Brasil**: seção 1, Brasília, DF, ano 2003, n. 174, p. 2-5, 9 set. 2003.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa n. 39, de 08 de agosto de 2007. Oficializa o método analítico para determinação da razão isotópica do carbono em suco, refresco, néctar e bebida de uva. **Diário Oficial da República Federativa do Brasil**: seção 1, Brasília, DF, ano 2007, n. 154, p. 3-3, 10 ago. 2007a.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Decreto n. 6.871, de 04 de junho de 2009. Regulamenta a Lei n. 8.918, de 14 de julho de 1994, que dispõe sobre a padronização, a classificação, o registro, a inspeção, a produção e a fiscalização de bebidas. **Diário Oficial da República Federativa do Brasil**: seção 1, Brasília, DF, ano 2009, n. 106, p. 20-29, 5 jun. 2009.

BRASIL. Agência Nacional de Vigilância Sanitária. Resolução RDC n. 5, de 15 de janeiro de 2007. Aprova o Regulamento Técnico sobre “Atribuição de Aditivos e seus Limites Máximos para a Categoria de Alimentos”. **Diário Oficial da República Federativa do Brasil**: seção 1, Brasília, DF, ano 2007, n. 12, p. 56-57, 17 jan. 2007b.

FIGUEIRA, R.; DUCATTI, C.; VENTURINI FILHO, W. G.; NOGUEIRA, A. M. P. Isotope analysis ($\delta^{13}C$) of pulpy whole apple juice. **Ciência e Tecnologia de Alimentos**, Campinas, v. 31, n. 3, p. 660-665, 2011a.

FIGUEIRA, R.; NOGUEIRA, A. M. P; DUCATTI, C.; VENTURINI FILHO, W. G.; MISCHAN, M. M.; SILVA, E. T. Método de análise isotópica ($\delta^{13}C$) e limite de legalidade em néctar de laranja. **Brazilian Journal of Food Technology**, Campinas, v. 14, n. 3, p. 211-219, 2011b.

FIGUEIRA, R.; NOGUEIRA, A. M. P; VENTURINI FILHO, W. G.; DUCATTI, C.; MISCHAN, M. M. Análise isotópica ($\delta^{13}C$) e legalidade em suco e polpa de caju. **Brazilian Journal of Food Technology**, Campinas, v. 14, n. 4, p. 317-324, 2011c.

JAMIN, E.; GONZALEZ, J.; BENGOECHEA, L.; KERNEUR, G.; REMAUD, G.; NAULET, N.; MARTIN, G. G. Measurement of $^{13}C/^{12}C$ ratios measured on sugars, malic and citric acids as authenticity probes of citrus juices and concentrates. **Journal of AOAC International**, Arlington, v. 81, n. 3, p. 604-609, 1998.

KELLY, S. D. Using stable isotope ratio mass spectrometry (IRMS) in food authentication and traceability. *In*: LEES, M. **Food authenticity and traceability**. London: Woodhead, 2003. chap. 7, p. 156-183.

NOGUEIRA, A. M. P.; FIGUEIRA, R.; DUCATTI, C.; VENTURINI FILHO, W. G. Análise isotópica ($\delta^{13}C$) para detecção de adulteração em néctares de pêssego. **Brazilian Journal of Food Technology**, Campinas, v. 14, n. 2, p. 115-124, 2011.

OLIVEIRA, A. C. B.; SALIMON, C. I., CALHEIROS, D. F.; FERNANDES, F. A.; VIEIRA, I.; CHARBEL, L. F.; PIRES, L. F.; SALOMÃO, M. S. M. B.; NOGUEIRA, S. F.; VIEIRA, S.; MOREIRA, M. Z.; MARTINELLI, L. A., CAMARGO**,** P. B. Isótopos estáveis e produção de bebidas: de onde vem o carbono que consumimos? **Ciência e Tecnologia de Alimentos**, Campinas, v. 22, n. 3, p. 285-288, 2002.

ROSSMANN, A. Determination of stable isotope ratios in food analysis. **Food Reviews International**, New York, v. 17, p. 347-81, 2001.

ROSSMANN, A.; KOZIET, J.; MARTIN, G. J.; DENNIS, M. J. Determination of the carbon-13 content of sugars and pulp from fruit juices by isotope-ratio mass spectrometry (internal reference method). A European interlaboratory comparison. **Analytica Chimica Acta,** Amsterdam, v. 340, n. 1/3, p. 21-29, 1997.