

## THE USE OF ISOTOPE RATIOS IN COMMERCIAL FRUIT JUICES AUTHENTICATION

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In this study, commercial fruit juices present on Romanian market were investigated, by mean of stable isotopes, in order to check their correct labeling. In order to detect the sugar addition, to investigated fruit juices,  $\delta^{13}\text{C}$  values of: whole juice, pulp and sugars were determined. Beside these,  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values of fruit juices water were measured in order to differentiate directly press juices from juices obtained from concentrates by re-dilution. The interpretation of these results is based on the fact that authentic juices have elevated  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  content of water as compared to water from re-diluted products, which were made using tap water which is relatively, depleted in heavy oxygen and hydrogen isotopes.

*Key words:* stable isotopes, fruit juices, food authenticity.

### 1. INTRODUCTION

Stable isotope determinations have become valuable tools in authenticity control and origin determination of food, food ingredients and beverages, especially fruit juices and wine, and also for the identification of the natural or synthetic origin of flavorings. Very frequently used is the determination of the relative carbon isotope abundance, the  $\delta^{13}\text{C}$ -value, mainly because the biochemical background of carbon isotope fractionation in nature is well understood and because the methods for the determination of the  $\delta^{13}\text{C}$ -values are well established [1].

The  $^{13}\text{C}/^{12}\text{C}$  ratios in terrestrial plants fall into three categories which are associated with the pathways of carbon assimilation: the conventional (C3) pathway, the dicarboxylic acid (C4) pathway, and the pathway associated with crassulacean acid metabolism (CAM) [2]. Plants that take up  $\text{CO}_2$  according to the C3 photosynthetic pathway discriminate against  $^{13}\text{CO}_2$  to a greater extent than plants with the C4 pathway [3]. As a result, C3 plants contain approximately 14% less  $^{13}\text{C}$  than C4 plants.  $\delta^{13}\text{C}$  values for C3 plants range from  $-33\text{‰}$  to  $-22\text{‰}$ , with an average of  $-27\text{‰}$  vs. VPDB, the fruits being C3 plants. C4 plants range from  $-16\text{‰}$  to  $-10\text{‰}$ . Examples of common C4 plants include corn, sugar cane, and sorghum [4].

Because certain sweeteners such as cane, corn, and beet sugars cost less than freshly squeezed fruit juices, they could be added to the juice to illegally extend it in conjunction with the addition of groundwater. Juice that has been adulterated with these sweeteners and sold as 100% pure without the declared addition of these compounds on the label may result in prosecution by regulatory agencies. The isotopic composition of sugars and water depends on both plant variety and geographic origin. Thus, addition of these materials can often be detected by measuring isotope ratios of the fruit juice sugars and water. The  $^{13}\text{C}/^{12}\text{C}$  ratio of fruit juice sugars differs from that derived from cane and corn sugars. The difference originates from the way the sugars are synthesized by the plant. Sugar cane and corn belong to a group of plants that use a C4 Hatch-Slack pathway to synthesize sugars. Beet sugar and citrus plants use the C3 Calvin cycle. As a result, different amounts of  $^{13}\text{C}$  are found in products derived from C3 and C4 plants.

Measurements of the stable isotope ratios of hydrogen ( $^2\text{H}/^1\text{H}$ ) and oxygen ( $^{18}\text{O}/^{16}\text{O}$ ) are applicable for the characterization of geographical origin because they are strongly latitude dependent [5]. While water is the only source of hydrogen for photosynthesis, oxygen is taken by plants from several sources, from atmospheric oxygen and carbon dioxide and, mainly, from water pools from the soil. Consequently, the  $^2\text{H}$ - and  $^{18}\text{O}$ -contents of fruit juices reflect the geographical origin of the product [6].

Our study aims to check the correct labeling of some commercial fruit juices present on Romanian market with respect to the illegally watering or sugar addition. This work, is also in agreement with the implementation of Directive 2012/12/EU from 19 April 2012: “relating to fruit juices amend the specific provisions of Directive 2001/112/EC concerning the labeling of fruit juices and similar products to reflect the new rules on authorized ingredients, such as those pertaining to the addition of sugars, which are no longer authorized in fruit juices”.

## 2. EXPERIMENTAL

Twelve commercial juices available on Romanian market were investigated in this work. Sugars were separated and purified from fruit juices according to the method proposed by [7] meanwhile for pulp separation the method by [8] was applied. The measurements of  $\delta^{13}\text{C}$  from fruit juice, pulp and sugars were carried out on an Elemental Analyser (Flash EA1112 HT, Thermo Scientific), coupled with an isotope ratio mass-spectrometer IRMS (Delta V Advantage, Thermo Scientific). For the quality control of our analysis, three working standards were analyzed at the beginning of each sequence, than three replicas from each sample were measured. NBS-22 oil with a certified value of  $-30.03\text{‰}$  versus PDB (Pee Dee Belemnite) was used as standard. For oxygen-18 determination 5 ml of raw juice (neither centrifuged nor filtered) was equilibrated with  $\text{CO}_2$  for 15 hours

according to the CEN: ENV 13141:1997 method at  $25 \pm 0.1^{\circ} \text{C}$ . The carbon dioxide was then extracted and purified. The  $^{18}\text{O}$  isotopic content of the water samples were then analyzed using a stable isotope ratio mass spectrometer IRMS (Delta V Advantage, Thermo Scientific). For the hydrogen analysis a distiller under static vacuum was used with „Rittenberg trousers” on 2-3 ml of fruit juice, always with the quantitative recovery of the water. For  $\delta^2\text{H}$  the equipment used was a Liquid-Water Isotope Analyzer (DLT-100, Los Gatos Research). The isotopic values were calibrated against laboratory-used standards (working standard 1, with  $\delta^{18}\text{O} = -11.54 \pm 0.1\text{‰}$  and  $\delta^2\text{H} = -79.0 \pm 0.1\text{‰}$ ; working standard 2, with  $\delta^{18}\text{O} = -7.14 \pm 0.1\text{‰}$  and  $\delta^2\text{H} = -43.6 \pm 1\text{‰}$ ; working standard 3, with  $\delta^{18}\text{O} = -2.96 \pm 0.1\text{‰}$  and  $\delta^2\text{H} = -9.8 \pm 1\text{‰}$ ).

### 3. RESULTS AND DISCUSSION

Authentic products present a natural variation of their stable isotope content mainly due to seasonal or regional influences. In many cases this variation is sufficiently wide to cover a small amount of adulteration which could remain undetected. One way to overcome this type of small adulteration is to use an internal reference approach by measuring the isotopic composition of two chemical components aroused from the same sample and to study their relationship in order to determine the authenticity [8, 9]. Rossmann *et al.* [10] proposed for the detection of exogenous sugars in fruit juices an internal reference method for the comparison of  $^{13}\text{C}$  content of sugars with those of insoluble component of a juice, the pulp. In this case the  $\delta^{13}\text{C}$  content of sugars and pulp will be in a constant ratio because they arise from closely related metabolic pools and were subjected to the same environmental influences [10]. Thus, the isotopic content of the reference compound is used to reduce the variability of authentic database and also to detect relatively small amounts of sugar addition even when the isotopic values of investigated sample are in the natural variation range of the fruits. Accordingly, this study established for orange juices the limiting value for the difference between sugar and pulp for the detection of exogenous cane sugars to be 1.25‰, meanwhile for grapefruit juices is 1.04‰.

In this work, to detect the sugar addition to investigated fruit juices, analysis of carbon isotopic ratios of: whole juice, sugars and pulp were performed. By determining the isotopic ratios  $^{13}\text{C}/^{12}\text{C}$  from whole juice ( $\delta^{13}\text{C}_{\text{juice}}$ ), the addition of  $\text{C}_4$  sugar type was clearly observed for samples: 3, 8 and 11 (Table 1). For sample 4 the corresponding value of whole juice  $\delta^{13}\text{C}_{\text{juice}} = -22.6\text{‰}$  indicate the present of  $\text{C}_4$  sugar, confirmed by the difference between  $\delta^{13}\text{C}_{\text{pulp}}$  and  $\delta^{13}\text{C}_{\text{sugar}}$  which is around -4‰. The presence of exogenous sugar, according to the labels, was also observed for samples 6 and 7 where differences between  $\delta^{13}\text{C}_{\text{pulp}}$  and  $\delta^{13}\text{C}_{\text{sugar}}$  were 1.6‰ and 1.8‰, respectively.

Table 1

Isotopic composition of investigated commercial fruit juices

No.	Sample	$\delta^{13}\text{C}_{\text{juice}}$ [‰]	$\delta^{13}\text{C}_{\text{pulp}}$ [‰]	$\delta^{13}\text{C}_{\text{sugar}}$ [‰]	$\delta^{18}\text{O}$ [‰]	$\delta^2\text{H}$ [‰]
1.	Apple juice directly press	-25.7	-27.5	-25.6	-2.9	-46.8
2.	Apple, carrot juice	-26.2	-26.4	-26.0	-8.7	-76.9
3.	Peaces juice	-16.8	-24.5	-16.4	-8.8	-62.0
4.	Apple juice	-22.6	-25.6	-21.7	-10.8	-84.2
5.	Apricot juice	-25.1	-25.2	-24.9	-8.8	-76.3
6.	Peaces juice	-25.7	-26.7	-25.1	-9.4	-75.7
7.	Tomato juice	-27.2	-28.3	-26.5	-10.6	-78.8
8.	Pear juice	-20.4	-26.7	-18.7	-7.2	-54.5
9.	Orange juice	-26.6	-26.8	-26.3	-8.4	-62.7
10.	Orange juice	-25.7	-25.1	-25.7	-6.7	-50.7
11.	Orange, mango juice	-17.4	-25.0	-17.3	-9.4	-76.2
12.	Orange juice	-26.5	-26.5	-24.7	-5.4	-45.3

For the directly press apple juice (sample 1), even if the  $\delta^{13}\text{C}_{\text{juice}}$  corresponding to the whole juice would indicate an authentic juice, the correlation between this value and those for  $\delta^{13}\text{C}_{\text{pulp}}$  and  $\delta^{13}\text{C}_{\text{sugar}}$  are not consistent with those reported by Lee and Wrolstad [11] for authentic apple juices. They reported that in the case of authentic apple fruit juices the  $\delta^{13}\text{C}$  value of the fruit pulp was found to be the same as those for the corresponding whole juices meanwhile sugars had slightly more positive  $\delta^{13}\text{C}$  values than the whole juices [12]. For sample 1, the differences between pulp and whole juice or between pulp and sugars are around 2‰, fact that suggest the presence of a small quantity of exogenous sugar. In contrast, for samples 2, 5, 9 and 10 small differences between  $\delta^{13}\text{C}_{\text{pulp}}$  and  $\delta^{13}\text{C}_{\text{sugar}}$  were observed (<0.6 ‰), these being in agreement with the results reported for authentic juices. Moreover, for samples 2, 5 and 9 the values for  $\delta^{13}\text{C}_{\text{juice}}$  (corresponding to the whole juice) have almost similar values as those for  $\delta^{13}\text{C}_{\text{pulp}}$ , while for sugars ( $\delta^{13}\text{C}_{\text{sugar}}$ ) are slightly more positive. The last investigated orange juice (sample 12) exceed the limiting value for the difference between sugar and pulp founded by Rossmann (1.25‰), being in our case 1.8‰, fact that suggests the presence of added sugar [10].

Stable isotope ratio measurements of oxygen and hydrogen ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ ) from fruit juices water are currently used to differentiate the directly press juices from those obtained from concentrates re-diluted with water. The common practice of fruit juices producing is to concentrate pressed juices for easier and cheaper storage and then dilute with tap water just before packaging [13]. For investigated samples, on the bases of our previously reported results [14–16] and also on those from literature [13] we can state that, except the sample 1, all samples were reconstituted from concentrates by re-dilution with tap water. These results were, however, consistent with the information provided on their label.

#### 4. CONCLUSIONS

$^{13}\text{C}/^{12}\text{C}$ ,  $^2\text{H}/^1\text{H}$  and  $^{18}\text{O}/^{16}\text{O}$  stable isotope ratios of 12 commercial fruit juices present on Romanian market were investigated in order to detect the presence exogenous water and added sugar. It was observed that except the sample 1 all fruit juices were obtained from concentrates re-diluted with water. Also, C stable isotope ratio from: whole juice, pulp and sugars were determined in order to detect the sugar addition. The presence of sugars coming from C4 plants (cane or corn) in samples 3, 4, 8 and 11 was detected directly from the analysis of carbon isotope ratio in whole juices. The measurements and the comparison of  $\delta^{13}\text{C}$  from sugars and pulp allow us to detect the presence of exogenous sugars also in samples: 1, 6, 7 and 11.

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