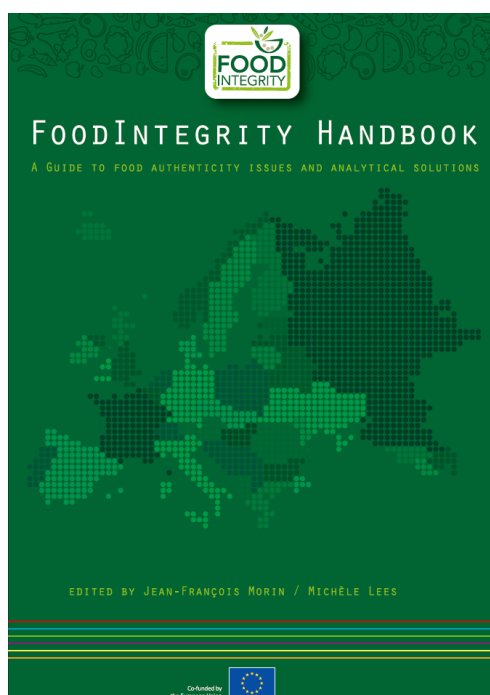


# FOODINTEGRITY HANDBOOK

## *A GUIDE TO FOOD AUTHENTICITY ISSUES AND ANALYTICAL SOLUTIONS*

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# Plant-derived sugars and sweeteners

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## General overview of the products

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The products covered in this chapter are all plant extracts that contain varying levels of sugars. The sugar contents vary from very low in the tree waters {saps} (ca 0.5 to 3 %) to more typical values seen in concentrated sugar syrups like liquid sucrose, invert, glucose or high fructose corn syrups (ca 65 to 70 %) that are found in maple, birch, coconut blossom and agave syrups. Bulk sucrose can also be prepared from two sources either cane or beet.

Over the last few years, due to all the bad publicity about consumption of sugary beverages, there has been a drive to produce low sugar beverages using natural extracts. This has led to the increased popularity of vegetable juices, fruit and vegetable juice blends and of course in the increased sales of coconut water. All these beverages typically have sugar contents between ca 4 to 7 %, which is still considered high by some consumers and has led to an interest in “tree” waters which have been a recent addition to the beverage market.

These products typically have much lower sugar contents being less than 3 % but are supposed to contain useful levels of amino acids, minerals and vitamins. However, the actual levels quoted on nutritional labels are below the values that would give any significant effect and no formal claims could be made in the EU. It will be interesting to see if there is a prolonged interest in this latter class of products, but one thing is sure if there any issues with authenticity in this area it would have a very detrimental effect on the sector.

As in many aspects of food there has been a consumer drive to what they consider more “natural” sweeteners and away from refined sugar and particularly high fructose corn syrup, due to all of the adverse publicity there has been around this product and its possible link to increased rates of obesity, whether the link is true or not. This has led to more interest in other sugar sources and there has been a steady growth in maple syrup production in Canada over the last five years. There has also been considerable interest in syrups produced from agave as an alternative sweetener.

Canada is the major source of maple syrup with a production of ca ten million US gallons (ca 38 million litres) in 2014. Quebec province is by far the largest producer within Canada and this province accounts for some 80 to 90 % of the country’s production. Figure 1 shows the seasonal changes in production volumes of maple syrup products (syrup and taffy) from Quebec over the period 1997 to 2014 [1]. As would be expected with a natural product yields vary from one year to the next, due to weather conditions in the spring and during the previous year’s autumn when the starch is being laid down for next year’s early season growth. However, there has been a general increase in production volumes, which has been achieved by an increase in “tapped” acreage and

also the addition of more “taps” to the recent plantings, which has become possible as these new trees mature and this allows more taps to be added to each tree.

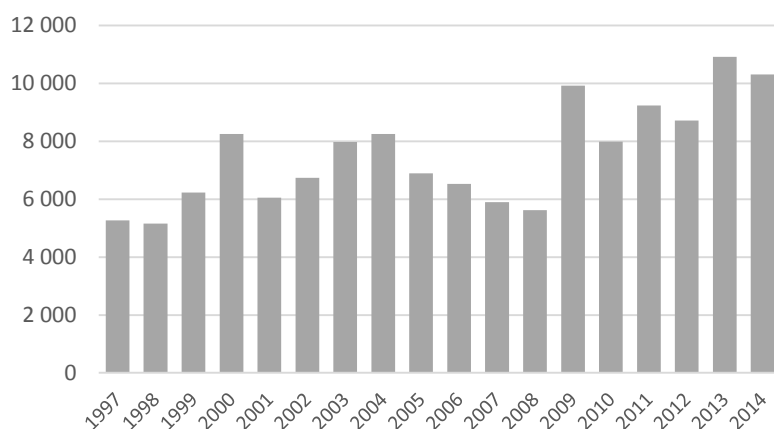


Figure 1: Maple syrup production data for Quebec province in 1 000's of US gallons from 1997 to 2014

## 1. Product Identity

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### 1.1. Definition of the product and manufacturing process

The products covered in this chapter are all sugar containing extracts produced from plants. For the purpose of this chapter they will be divided into a number of classes.

**“Tree” waters:** The products discussed here are produced from the sap collected from a tree in the early spring. The liquid is subjected to little additional treatment; apart from processes to limit microbiological changes e.g. ultrafiltration and/or pasteurisation. Two products will be considered here which are extracted from maple or birch trees. There are other “tree” waters like bamboo, but this will not be covered here due to a lack of background information on the product. However, the authenticity issues identified for maple and birch waters may also be applicable to this material.

**“Tree” syrups:** These products are also produced from tree saps but are concentrated by simple heat or using a pre-concentration step {such as ultrafiltration or freeze concentration} followed by heating to remove most of the water. The most common of these tree syrups is derived from maple sap and the syrup typically has a Brix (sugar) content around 66 %. The former is often used as a topping for pancakes and waffles for instance, whereas birch syrup is often used more in savoury dishes.

Another type of “tree” syrup is extracted from coconut flower spikes. Unlike the true tree syrups, such as maple, this material is actually extracted from the flower spike from which the coconuts themselves will develop later. Once the sap is collected the water is again removed from the sap by heating to give either a syrup (*ca* 65 %) or coconut sugar if the Brix is increased to a higher level that promotes crystallisation of the sugars on cooling.

**Agave syrups:** These products are syrups derived from a range of succulents of the “agave” family (e.g. *americana*, *tequilana* and *salmiana*). These syrups are produced by the extraction of the storage carbohydrate (inulin) from the agave pinas and its subsequent hydrolysis to liberate a fructose rich syrup which is then concentrated to *ca* 70 Brix.

**Plant derived sugars:** Bulk sucrose can be prepared from two sources either cane or beet. The former is produced in hot climates like Brazil and the Caribbean whereas beet sucrose is produced in more temperate climates such as Northern Europe, USA, Canada, Russia and Turkey.

## 1.2. Current standards of identity or related legislation

Many food products are defined by a set of specifications which may be regulated or may be found in industry sector guidelines. These are often used as the basis for highlighting deviating composition or fraudulent practices.

### 1.2.1. Tree waters

Although maple syrup has been produced for centuries, originally being exploited by the indigenous inhabitants of the North American continent, the sale of “maple water” is a relatively new product addition.

At present there are no National or International standards for tree waters. Although the defining criteria used in the Canadian standard for maple syrup [2] could be applied to maple water, with the exception of the concentration factor. “Maple water” is the sap extracted from the trees of the *Acer* family and nothing should be added in the way of colours, acids, antifoam agents or preservatives. There is one industry initiative in Canada (NAPSI) to control the quality and authenticity of maple water, which is run by maple syrup producers of Quebec [3]. This standard defines minimum quality standards and involves both inspections of production facilities to ensure that they comply with the standard and analysis of the final product to ensure that production batches meets the industry’s quality criteria. At present there are no industry initiatives to define birch water.

### 1.2.2. Maple syrup

This is a product that most people will be aware of; it is a syrupy brown product with a pleasant sweet and a rather unique taste.

Unlike the tree waters, there are both Canadian [2] and US [4] standards that define “maple syrup”. There are also some regional standards that have been developed by the producer provinces in Canada and in US States such as Vermont [5]. Unlike the US standard, that allows the addition of certain additives that may enhance the taste (salt), microbiological stability (preservatives) or processability (anti-foaming agents) of the product, the Canadian and Vermont standards prohibit the addition of these additives and the syrup has to be 100 % derived from maple sap.

As would be expected the two National standards are basically very similar but the US standard allows more flexibility [4], as described above, where some additives are allowed but would have to be detailed in the ingredients list, when added. There are no worldwide (Codex) or European (EU) standards for maple syrup.

By convention, US and Canadian Standards, the minimum Brix that is acceptable for this product is 66 %. Unlike Birch syrup the majority of the Brix in maple syrup is sucrose (98 to 99 %) with only very low levels (< 1 %) of glucose and fructose being detected.

### 1.2.3. Coconut blossom (flower) syrup and sugar

As with maple syrup, to produce coconut blossom (flower) syrup the tree or this case the palm has to be tapped to allow extraction of the sap. However, unlike maple and birch syrups, the palm (*Cocos nucifera*) itself is not tapped but a cut is made in the flower spike that will eventually carry the coconuts, hence the term blossom or flower syrup/sugar (see Figure 2). It is typically produced in coastal areas of the Indian and Pacific Oceans, with the major suppliers in Thailand, Indonesia, and the Philippines.

Although there is no International standard (Codex) for coconut flower syrup/sugar, the Philippine Government has prepared a standard for coconut flower sugar [6], but not the syrup. The standard details how the product is produced and contains a few physiochemical properties of the sugar like: typical sugar levels (sucrose, glucose and fructose), water activity, ash content and some visual, taste and odour characteristics. It also lays down maximum levels for a range of micro-organisms including the pathogens: *Salmonella*, *E. coli* and coliforms. It also provides the criteria by which the quality of the product can be judged (premium, class I and class II), from the colour of the sugar and the product's water activity. Although this is a Philippine standard, it seems to be fairly well accepted as a quality standard in a number of countries in Asia.



Figure 2: Palm flower spike being cut prior to collection of sap for coconut sugar production

### 1.2.4. Palm syrup/sugar

Similar products can also be extracted from palms around the Indian and Pacific oceans. In areas of Africa, Asia and New Guinea the palmyra palm (*Borassus* spp.) is generally used. In the Middle East and the Mediterranean region the date palm (*Phoenix dactylifera*) is generally used, whereas *P. sylvestris* is the more common source of date sugar in parts of Asia like Pakistan and India. Date

sugar is an additional product from this plant as most of these palms are grown for date production. The nipa palm (*Nypa fruticans*) is also commonly used for syrup production on the coastlines and tropical regions of the Indian and Pacific Oceans. It is the only palm that will tolerate high water levels, as seen in mangrove swamps, where often only its leaves and flowers are seen above the water level. Finally, the sugar palm (*Arenga pinnata*) can be used in the coastal and tropical regions of Asia, around China and Indonesia for syrup/sugar production. The sugars are all derived from a tap on the flower spikes of these plants and are analogous to coconut blossom syrup/sugar. There is no standard for these products.

### 1.2.5. Agave syrup

Although the syrup from *Agave salmiana* can be produced in a similar manner to that employed in palm saps, it can also be processed as given for *Agave tequilana*. In the former case as the agave plant matures it produces a stork in the centre of the plant and if this is truncated the sap will flow slowly into the remaining hole and can be collected daily. The liquid is heated to breakdown the complex carbohydrates to form simple sugars fructose, glucose and small amounts of sucrose.

To produce premium tequilas, *Agave tequilana* should be used as the only sugar source, likewise for premium Mezcal products, only sugars extracted from *Agave salmiana* should be employed.

However, when it comes to agave syrup production there is more flexibility allowed in the selection of permitted species that may be used to produce the syrup. Here it is much less defined and in fact many related species are used here due to:

- a) the high price of *Agave tequilana* and *Agave salmiana* pinas,
- b) lack of supply for these two types of pinas due to competition from spirit producers

Pinas, as shown in Figure 3, from many different agave species are used in syrup production to reduce costs.



Figure 3: Agave “pinas” after leaf stripping showing relative size (ca 40 to 60 kg)

The finalised agave syrup will be a clear pale yellow/yellow product with a Brix of *ca* 70 %. The majority of the soluble solids of these products are the simple sugars, fructose and glucose, which are the major components of inulin. Typically, the fructose to glucose ratio is around 10.0 in these materials. Sucrose and other disaccharides are also present in the syrup, but at low concentrations, with variable levels of the polyols mannitol and inositol are also seen.

Although there is no International standard for agave syrup, Mexico published a non-binding standard (NMX) for this material in 2008 [7] as a preliminary step towards the introduction of a binding/mandatory norm (NOM), which was adopted in 2016 [8]. Since then, any agave syrup exported from Mexico has to comply with this standard.

### 1.2.6. Bulk sucrose production

Although there is a Codex [9] international standard for different qualities of sugar, e.g. white, soft white, soft brown, dextrose, glucose syrup and raw cane sugar and one for the EU [10], these only define some basic physicochemical parameters that allows the product to be placed in the correct category. These standards do not differentiate between white sugar, sucrose, prepared from beet or cane.

## 2. Authenticity issues

### 2.1. Identification of current authenticity issues

These products, along with most fruit juices and honey, are sold on the basis of their sugar content (Brix). This presents an opportunity for an unscrupulous supplier to adulterate their products as there are always other cheaper sugar sources than can be used as a substitute for the sugar materials extracted from the plant (tree, palm or succulent). This substitution has been found to occur from time to time and some examples of these will be detailed here. Presented in Figure 4 are typical prices for the adulterants that have been used to extend these types of products together with the costs for the authentic materials (where prices for bulk purchases are available).

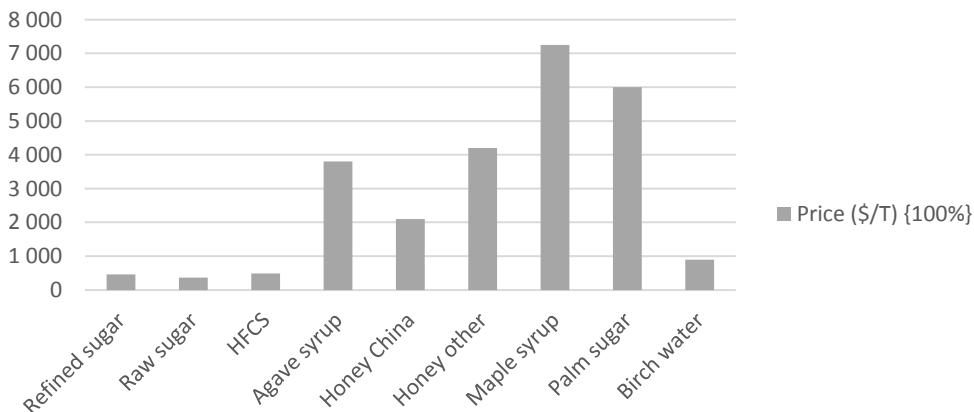


Figure 4: Nominal prices for “real” products and potential adulterants in 2016

Due to the high difference in price between the potential adulterant and the authentic vegetable sugars (a ratio of 1 to 10), the relative additional profit that an unscrupulous supplier can make by extending the authentic material by 20 % with an appropriate type of sugar is clear so there is a significant driving force if someone wants to cheat.

The typical materials that have been used to extend these products are cane and/or beet sucrose, high fructose syrups from starch (corn and/or rice), invert syrups from cane or beet and unrefined sugar.

The material selected to carry out the adulteration has depended on:

- a) availability of another cheaper sugar source,
- b) the knowledge and sophistication of the adulterator,
- c) surveillance operations that have taken place on this product.

The substitution of part or all of maple syrup with cheaper materials is an issue that occurs from time to time. Recently the maple syrup producers of nine US producer states have petitioned the FDA to look at a number of products that appear to them as misrepresenting their contents [7,8]. Under US law only products containing a minimum of 10 % maple syrup can use the term “Maple” in their name. The FDA subsequently published a “note to consumers” to advise them to study the label of products carefully so they were not misled [11].

There have also been other cases in the US where products containing very little maple syrup present themselves in the manner in which they are marketed as if they contain a higher level of maple syrup [12]. In a study published in 2010 the authors [13] looked at reported cases of adulterations of food from 1980 to 2010 and found that there were 16 cases of the adulteration of maple syrup over that period, which represented *ca* 2 % of all of the examples they had examined.

Around a similar time, there was a small-scale study carried out in the UK on palm sugar. This identified that there were samples of this sugar on sale in the UK which were misrepresented as they were blends of palm sugar and cane/corn sugars. Here  $\delta^{13}\text{C}$  values were shown to be in the region of -13 to -15 ‰ [14], which clearly indicated that the majority of the sugar was actually derived from cane/corn rather than being isolated from the palm.

One reason for the publication of the Mexican standard for agave syrup was the relatively high number of cases of syrup detected in both Europe and the US that clearly showed the presence of sugars derived from other sources (cane and corn) rather than being fully derived from agave [15].

The authors are unaware of any published examples of the sale of adulterated maple or birch waters but there is certainly a risk that these products could be extended by unscrupulous suppliers.

## 2.2. Potential threat to public health

So far the extension of any of the products covered in this chapter have not presented a risk to health, as their adulteration has involved the substitution of the soluble solids extracted from the appropriate plant with other “food grade” sugar materials extracted from another plant, but which had a lower cost. However, this does not preclude that the adulteration of these products in the future will always present no risk to health.



## 3. Analytical methods used to test for authenticity

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### 3.1. Officially recognised methods

#### 3.1.1. Major sugars composition

One way to assess if these products look “normal” is to determine the levels of the simple sugars (sucrose, glucose and fructose) they contain. In some cases, this can be very useful, for instance if high levels of glucose and fructose relative to sucrose are detected in a maple syrup/sap or palm flower sugar/syrup. This should instantly arouse suspicion that either invert syrup or a high fructose corn syrup has been used to extend (dilute) the product.

There are many ways that the sugars can be determined either using an ICUMSA method (International Commission for Uniform Methods of Sugar Analysis) [16] or an AOAC procedure [17]. Most of the methods are based on some form of chromatography using either refractive index or electrochemical detection procedures. The chromatography is either based on an amino bonded column using water/acetonitrile as a solvent or a mixed bed resin, such as a Dionex PA-1, using aqueous sodium hydroxide as an eluent. Some of the papers mentioned above for the analysis of these materials have involved the analysis of sugars using gas chromatography and flame ionisation detection (GC-FID). This procedure involves freeze drying and derivatisation of the sugars, which is time consuming but is still a possibility, prior to the separation of the derivatives on a GC column (typically a non-polar e.g. DB5 or similar) [18].

However, if an unscrupulous producer “knows” what they are doing they will carefully select the correct “type” of sugar to extend their products, which means that using a simple sugars analysis will not allow the detection of the adulteration. Therefore other methods have to be employed.

#### 3.1.2. Isotopic analysis

One topic that is of significance to help control the authenticity of these products is the use of isotopic methods. These methods rely on the measurement of the small differences in the levels of the stable isotopes ( $^2\text{H}/^1\text{H}$ ,  $^{13}\text{C}/^{12}\text{C}$ ,  $^{18}\text{O}/^{16}\text{O}$ ) seen in a product. This allows the detection of the economic adulteration of these products by the addition of exogenous sugars.

In nature there are three different pathways that plants fix carbon dioxide from the atmosphere and these give rise to slightly different levels of the carbon isotopes seen in the sugars and other components of the plant.

- 1) **C<sub>3</sub> (photosynthetic) pathway:** Most plants use this pathway to fix carbon dioxide from the atmosphere. Here there is a large difference in the rate of reaction of  $^{13}\text{CO}_2$  and  $^{12}\text{CO}_2$  during fixation, which means less of the heavy form of carbon, is incorporated into the plant. This gives rise to carbon isotope ratios ( $\delta^{13}\text{C}$ ) in the region of -25 ‰ (parts per thousand) for the sugars and acids extracted from these plants. Most fruits and all tree saps fit in this class.
- 2) **C<sub>4</sub> (Hatch Slack) pathway:** Plants that use this pathway show a smaller isotope effect as the rate limiting step is not the actual fixation of the gas from the atmosphere, which it is in the C<sub>3</sub> route. This means that the sugar and acids in these plants show a higher level of the heavy carbon isotope and so show less negative values for their isotope ratio ( $\delta^{13}\text{C}$ ). Products in this category have  $\delta^{13}\text{C}$  values around -10 to -11 ‰. Plants of interest here are cane and corn, which are often one of the major adulterants used to extend sugar rich products like juices and the products covered in this chapter.

- 3) **CAM (Crassulacean Acid Metabolism) pathway:** The final route is used by a range of specialist plants that have to limit water loss during the day. This means that they shut their stomata, holes in the underside of their leaves, through which they absorb carbon dioxide during the day and so it is fixed at night. In these plants the isotope ratio ( $\delta^{13}\text{C}$ ) is typically between -12 and -15 ‰. Plants in this category are succulents mainly, e.g. pineapples and agave.

Plants also accumulate different levels of hydrogen ( $^2\text{H}/^1\text{H}$ ) and oxygen isotopes ( $^{18}\text{O}/^{16}\text{O}$ ) dependant on climatic and geographic conditions. The use of ( $^2\text{H}/^1\text{H}$ ) sometimes allows the differentiation of natural sugars from exogenous sugars, derived from a  $\text{C}_3$  source (beet/rice), that have been added to extend a product. The hydrogen isotopic pair together with oxygen isotopes has also been used to help differentiate between not from concentrate (NFC) and from concentrate fruit juices (FC).

All tree and palm saps are extracted from plants that use the  $\text{C}_3$  pathway and so show  $\delta^{13}\text{C}$  ca -25 ‰. Therefore the use of carbon isotopic analysis allows the addition of cane sucrose, cane invert and corn-based syrups (glucose and HFCS) to be detected when it is added to maple syrup/sap/sugar and coconut flower sugar/syrup at a level of ca 10 % and higher. This sort of detection level is lower than would be possible when using just conventional sugar analysis which has to allow for the natural variation in the glucose and fructose levels seen in these products. However, as agave is a succulent it uses the CAM pathway to fix carbon dioxide and means that standard carbon isotopic analysis is not sensitive enough to differentiate between 100 % agave syrups and materials adulterated with corn syrups.

There are two methods that can be applied here that have been validated on maple syrup. The first of these, AOAC 984.23 [19] {"whole sample method"}. This uses simple combustion of the sample to carbon dioxide which is then analysed by isotope ratio mass spectrometry. Although the AOAC method uses an off-line combustion of the sample and then introduction of the carbon dioxide produced into the mass spectrometer, a continuous flow system is now normally used where each sample is combusted in turn and the liberated carbon dioxide is directly fed into an isotope ratio mass spectrometer (IRMS) by a stream of carrier gas ( $\text{N}_2$ ) (EA-IRMS).

The second method {"ethanol method"}, AOAC 2004.01, uses a similar detection method but involves the initial fermentation of the sugars into ethanol ( $\text{EtOH}$ ), which is then recovered by careful distillation using a spinning band column. The ethanol is then subjected to  $^{13}\text{C}$  isotopic analysis using an IRMS machine [20]. The two methods have similar sensitivities, with a detection limit in the region of a 10 % addition of a  $\text{C}_4$ -derived sugar but give rise to very different  $\delta^{13}\text{C}$  values. This difference arises from the loss of some carbon atoms, as carbon dioxide, during the fermentation step, which means that it is critical that the correct "judgement criteria" are used to assess a sample e.g. the judgement criteria must be ones developed using the same analysis procedure e.g. "whole sample" or "ethanol" procedure.

The sensitivity of the  $^{13}\text{C}$  IRMS method for the detection of added  $\text{C}_4$  sugar to maple syrup/sugar can be improved by using malic acid as an internal standard [21]. Here the  $\delta^{13}\text{C}$  values detected for the sugars and the malic acid isolated from the sample are compared. A similar improvement in sensitivity of the detection of added  $\text{C}_4$  sugars to palm sugar has been published by Kelly [22]. This uses a similar approach to that given in the "internal standard" method published by AOAC (998.12) [23]. Here the protein contained in the sample is precipitated and washed to remove any bound sugars. After drying this material is then subjected to combustion and the  $\delta^{13}\text{C}$  ratio is determined using IRMS. This value is then compared with the  $\delta^{13}\text{C}$  value obtained on the "sugar" portion or on the whole sample, with no pre-treatment. These two values should be close to each.

If the percentage of C<sub>4</sub> sugars calculated using equation (1), as defined in the AOAC method, is larger than 7 % then the sample can be considered as adulterated with a C<sub>4</sub> sugar source.

$$C_4 \text{ sugars } \% = \frac{\delta^{13}C_p - \delta^{13}C_s}{\delta^{13}C_p - (-9.7)} \times 100 \quad (1)$$

$\delta^{13}C_p = {}^{13}C$  ratio seen in “protein” fraction,  $\delta^{13}C_s = {}^{13}C$  ratio seen in the sugar

The addition of cane sugar or HFCS to agave syrup (CAM) cannot easily be detected by <sup>13</sup>C-IRMS as the three plants share similar global  $\delta^{13}C$  values around -11 to -12 ‰. This means that other routes must be applied to detect this type of adulteration, which will be discussed in Section 3.2.

If a C<sub>4</sub> sugar (sucrose or cane invert), is added to a tree sap derived material, the C<sub>4</sub> derived sugars are concentrated in one of the components (either sucrose or glucose and fructose respectively) and this causes a disturbance in the carbon isotope ratios and means that the detection level is roughly halved over the global EA-IRMS approach. In work carried out using liquid chromatography linked with elemental analysis and isotope ratio mass spectrometry LC-EA-IRMS on honey [24] and agave syrups it has been reported that the carbon isotope ratios for glucose and fructose were much closer, within 0.5 ‰ for agave syrups, than seen in fruit juices, suggesting that this route offers a method to detect the adulteration of agave syrups with C<sub>4</sub> derived sugars. However due to the complexity and the low implementation of this hyphenated technique in food control laboratories, even if the intra-lab uncertainty of this technique is similar to the current EA-IRMS technique, the inter-lab uncertainty is still higher at the date of this publication.

The methods discussed above only address the addition of C<sub>4</sub> sugars to products derived from tree saps (maple, birch and palm products {syrup/sugar/sap}). However, if the unscrupulous supplier uses beet sucrose, which is derived from a C<sub>3</sub> plant, there will be no derivation in the  $\delta^{13}C$  isotope ratio as seen with cane sucrose. Here detecting this type of adulteration requires a different method.

The level of deuterium at the methyl site (D/H)<sub>1</sub> of the EtOH, produced during the fermentation of the sugars in the maple syrup, has been found to be very useful. The analysis is carried out using Deuterium-Nuclear Magnetic Resonance (<sup>2</sup>H-NMR) spectroscopy. The EtOH is again recovered from the fermentation broth by careful distillation using a spinning band column as mentioned above for <sup>13</sup>C measurements. In fact, if when employing this SNIF-NMR® method the EtOH-IRMS method is also used, it is possible to detect added exogenous sugars derived from both C<sub>3</sub> and C<sub>4</sub> sources. This is described for maple syrup in the AOAC Official method 2000.19 [25]. By combining <sup>2</sup>H-SNIF-NMR® and IRMS on the ethanol probe, it is therefore possible to detect addition of both beet and cane sugars/syrup in C<sub>3</sub> syrups such as maple as illustrated in Figure 5 below.

Although not normally used in this sense, the <sup>13</sup>C-IRMS and SNIF-NMR® methods can be used to determine whether sucrose is correctly described as “cane” or “beet”. In the former case for pure cane sucrose the  $\delta^{13}C$  value would be ca -11 ‰ and the (D/H)<sub>1</sub> value would be around 110 ppm, whereas if sucrose derived from beet was added much lower  $\delta^{13}C$  and (D/H)<sub>1</sub> values would be detected. Conversely with beet sucrose, if cane sucrose had been added to the product it would show a less negative  $\delta^{13}C$  value and the (D/H)<sub>1</sub> value would be higher. These two isotopic methods are the only two procedures that can be used to differentiate these two products. Similarly with starch derived syrups for which the corn or rice source can be differentiated from their  $\delta^{13}C$  values.

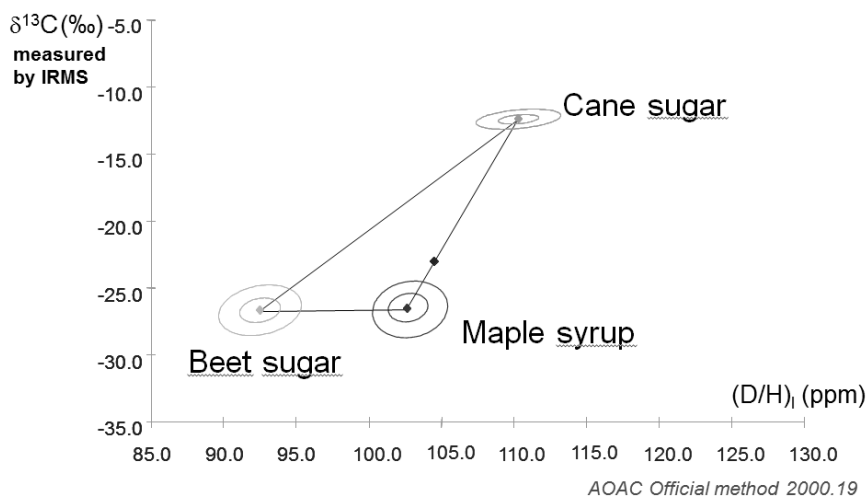


Figure 5: SNIF-NMR<sup>®</sup> plot for detection of C<sub>3</sub> and C<sub>4</sub> sugars to maple syrup

### 3.1.3. Mineral profile

Other components in the products can be measured such as minerals (K, Mg, Ca and Na) but the natural variation for these components can be rather large so unless there is a gross adulteration it will probably not be picked up using these components. Meanwhile it can be an additional analytical tool when comparing a suspect sample to a “witness” sample.

### 3.1.4. Minor sugars and sugar-alcohol profile

The Mexican standard for agave [7,8] defines a maximum level for sucrose/difruuctose and ranges for fructose, glucose and mannitol which are measured using HPLC. High pH anion exchange chromatography linked with electrochemical detection (HPAEC-PAD) can be used to measure the levels of mannitol and inositol in agave syrups to detect dilution from the addition of other sugar materials [15,26] using a Dionex MA-1 column.

Ideally a range of methods should be applied to a sample to ensure that there is the best chance of detecting the addition of any exogenous sugars.

## 3.2. Other commonly used methods

### 3.2.1. Sugar profile by Cap-GC

One approach that was not mentioned above but has proved very useful at detecting the addition of high fructose syrups derived from starch and cane/beet invert syrups is the use of Cap-GC. Here the syrups show marker peaks that are not seen in the sugars/syrups/saps and allow their detection at relatively low levels of addition (2 to 5 %). This is often more sensitive than any other procedure. The method was originally developed by Pr. Low of the University of Saskatchewan [27] to detect the addition of these same adulterants to fruit juices. It was subsequently extended to detect the addition of high fructose syrups derived from inulin to apple juice [28]. The procedure is detailed, with judgement criteria, in the International Fruit Juice Union’s recommendation # 4 [29].

Here the sugar/syrup/sap is freeze-dried to remove the water. The sugars are then derivatised using pyridine/trimethylsilylimidazole mixture (4:1) with heating. The samples are then injected on to a non-polar Capillary-GC column (DB 5) and the peaks are detected using a flame ionisation detector (FID).

Although high fructose syrups from starch (e.g. corn and rice) do not make good adulterants for maple or coconut flower syrups/sugar — as their addition will reduce the sucrose level while also increasing the levels of glucose and fructose — they have been used to extend these types of products because of their low price and large availability. If this type of material is added to maple syrup it is detected by the presence of maltose and isomaltose, as shown in Figure 6.

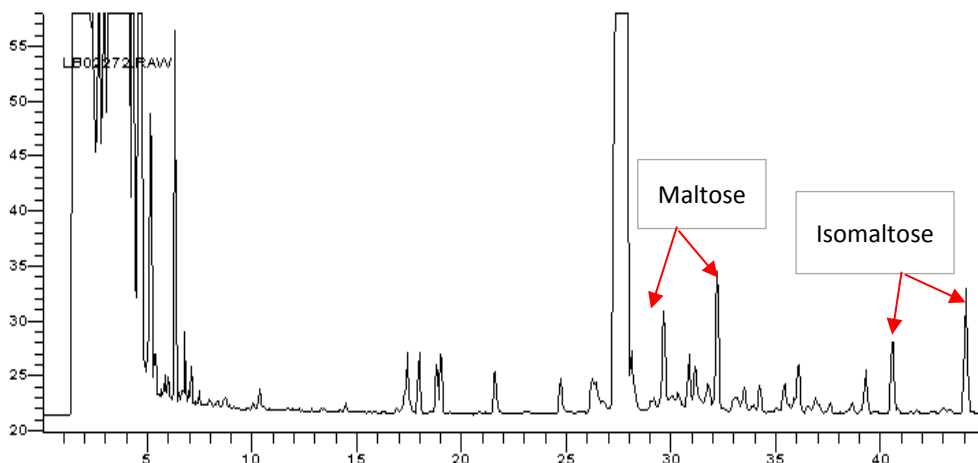


Figure 6: Cap-GC profile for maple syrup adulterated with HFCS

Here an addition of HFCS at a level of around 2 % is often detectable, which is much better than can be achieved using HPLC, to look at the simple sugar levels, or by  $^{13}\text{C}$ -IRMS. The Cap-GC procedure can also detect the presence of invert syrup when added to maple syrup/sugar and coconut flower syrup/sugar. Again there are two peaks that show the presence of the exogenous sugars in the product.

The Cap-GC method can also be used to analyse agave syrups for the addition of HFCS. Unlike the case of maple syrup, HFCS makes a “better” extender for agave syrup as they:

- a) Are rich in fructose, similar to agave syrups,
- b) Share a similar global  $\delta^{13}\text{C}$  value to that of agave as they are produced from a CAM plant.

Both features make them hard to detect by other analytical procedures.

When adulterated with HFCS, two signals corresponding to isomaltose appear in a flat zone, which are not present in typical Cap-GC profile for agave syrup (see Figure 7).

HPAEC-PAD can also be used to look for the presence of exogenous gluco-oligosaccharides that maybe present if a starch derived syrup is added to these syrups [26]. This uses a different column, with a lower retentivity (Dionex PA-100), to that used for the mannitol/inositol quantification discussed above. This method can be complementary to the Cap-GC method discussed above but looks at a different set of compounds.

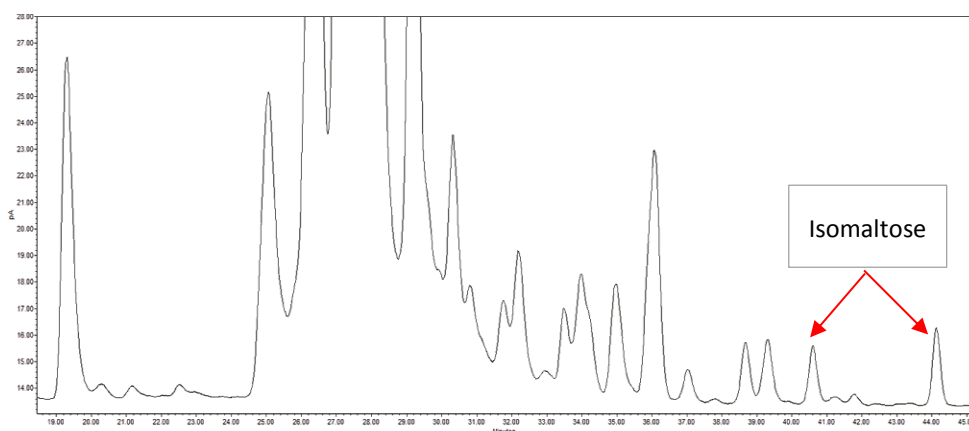


Figure 7: Portion of a Cap-GC profile for agave syrup adulterated with HFCS

Although the Cap-GC method offers one of the fastest and most sensitive routes to detect sugar syrup addition to these types of products, it will not detect the addition of either cane or beet sucrose to maple or coconut flower syrup. The former maybe detectable using  $^{13}\text{C}$ -IRMS, if the addition level is high enough, whereas beet sucrose addition can similarly be detected using SNIF-NMR<sup>®</sup>. This reinforces the need to use more than one method in screening samples to ensure any adulterations are detected.

### 3.2.2. Carbon 13 site specific natural isotopic fractionation

Another approach that has proved very useful to detect extension of agave syrups with cane and/or corn derived sugars is quantitative  $^{13}\text{C}$ -NMR ( $^{13}\text{C}$ -SNIF-NMR) [30]. Once again, the sugars are fermented into alcohol and recovered by careful distillation as per the SNIF-NMR<sup>®</sup> procedure. However, instead of using deuterium as a nucleus, carbon is used here, and the relative proportions of  $^{13}\text{C}$  at the  $\text{CH}_3$  and  $\text{CH}_2$  sites allows cane or corn syrups to be detected in agave. Although the global  $^{13}\text{C}$ -IRMS method does not show a significant difference between cane/corn & agave derived sugars, it has been found that the relative levels of  $^{13}\text{C}$  at the methyl and methylene sites of the EtOH show different levels of the heavy carbon isotope. This means that if  $^{13}\text{C}$ -NMR is used on the EtOH from these sources they can be differentiated, which is illustrated in Figure 8.

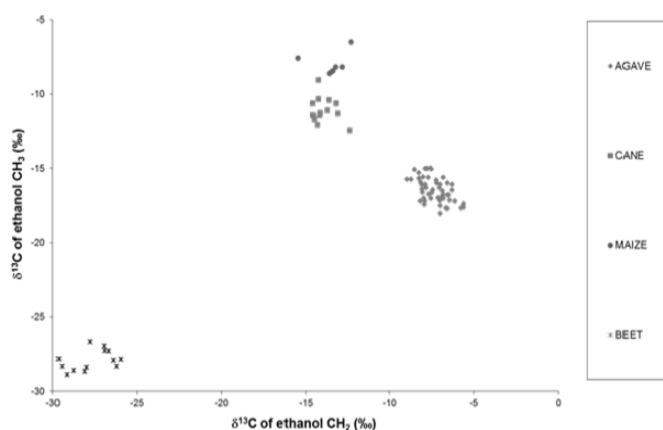


Figure 8: Plot of  $^{13}\text{C}$  site-specific values of ethanol detected using  $^{13}\text{C}$ -SNIF-NMR for agave and adulterants [30]

The detection limit for this technique to detect the presence of cane/corn sugars in agave syrup is around 15 %. This is much higher than is possible, in some cases, using Cap-GC for HFCS, however, as mentioned above cane invert is only readily detectable using this approach. Cane sucrose addition to agave could be detected by elevated levels of sucrose, which are relatively low in agave syrups. However, some agave syrups naturally contain low, but significant levels of, inulobiose (1-O- $\beta$ -D-fructofuranosyl-D-fructose) which may be seen to elute very close to sucrose on many chromatographic systems and caution needs to be taken in interpretation these results.

### 3.2.3. Organic acids screening

Some problems have been encountered with the addition of both citric and ascorbic acids to coconut water, which is another low acid product. Therefore some screening for organic acids in these products should be considered. Malic acid is the major acid seen in many of these products and there is an old validated procedure for this acid at AOAC (# 959.13) for checking the levels of malic in maple syrup, which would be better detected using a more modern HPLC method. This can be used to check for malic and citric acid in one run. Citric acid is often added to low acid products to “improve” their microbiological stability, by lowering the pH, and thus making it less susceptible to the growth of pathogenic micro-organisms. Although not validated on any of the matrices in this chapter, the HPLC-UV method (AOAC # 986.13) [31], would be suitable for malic and citric acids. However, different dilutions might be required to measure concentrations of both acids in these products.

### 3.2.4. Enzymatic methods

Although not commonly used in North America, there are several enzymatic methods for the quantification of both sugars and acids, which have been validated in Europe [32–39] for the analysis of fruit juices. These procedures would also be suitable for the analysis of the matrices covered in this chapter although they are not officially validated on any of these materials. The enzymatic methods offer one advantage in the analysis of malic acid as there are two different procedures for the determination of the two optical forms (L and D) of the acid. It was found in the past that often racemic D, L-malic acid was added to fruit juices, which could be detected using the D-malic assay [39]. If significant levels of this D-malic acid are detected the product is clearly adulterated.

## 4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
HPLC profile	Relative concentrations of simple sugars and sugar-alcohol (sucrose, glucose, fructose and mannitol*)	Detection of added sugar by distortion of sugar proportions & ratios
Atomic absorption spectroscopy or inductively coupled spectroscopy {MS or OES}	Concentrations of minerals (K, Mg, Ca, Mn and Zn)	Dilution of sugar syrup
HPLC-UV	Organic acids	Detection of the addition of citric acid and quantification of malic acid
Enzyme assay for D-malic acid	Organic acid, including optical active forms	Detection of the addition of racemic (D, L)-malic acid
Isotope ratio mass spectrometry (EA-IRMS)	Bulk $\delta^{13}\text{C}$ value	Addition of $\text{C}_4$ derived sugars to products <sup>§</sup>
Isotope ratio mass spectrometry (EA-IRMS, LC-IRMS)	Sugar and malic acid $\delta^{13}\text{C}$ values	Addition of $\text{C}_4$ derived sugars to products (refined procedure) <sup>§</sup>
Isotope ratio mass spectrometry (EA-IRMS, LC-IRMS)	Sugar and protein $\delta^{13}\text{C}$ values	Addition of $\text{C}_4$ derived sugars to products (refined procedure) <sup>#</sup>
Quantitative deuterium nuclear magnetic spectroscopy ( $^2\text{H}$ -SNIF-NMR)	Deuterium level at $\text{CH}_3$ site of EtOH (liberated by fermentation of sugars)	Detection of the addition of exogenous $\text{C}_3$ derived sugars
Quantitative carbon 13-nuclear magnetic spectroscopy ( $^{13}\text{C}$ -SNIF-NMR) <sup>®</sup>	Relative levels of $^{13}\text{C}$ isotope at $\text{CH}_3$ and $\text{CH}_2$ positions of EtOH (liberated by fermentation of sugars)	Detection of the addition of added cane/corn sugars to agave syrup <sup>£</sup>
High pH anion exchange chromatography linked with electrochemical detection (HPAEC-PAD)	Presence of gluco-oligosaccharides	Addition of starch derived syrups
High pH anion exchange chromatography linked with electrochemical detection (HPAEC-PAD)	Levels of mannitol and inositol	Detection of dilution of agave syrup
Capillary Gas-Chromatography (Cap-GC)	Presence of marker disaccharides for sugar syrups	Addition of exogenous sugar syrups (from starch (glucose and HFS) & invert syrup)

\* Levels are defined in Mexican Standard for agave syrups. § excludes agave syrup. £ not applicable to maple, birch and coconut flower syrups/sugars/saps. # applicable to coconut flower sugar



## 5. Conclusion

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The main component of all the products covered in this chapter is sugar. Unfortunately as there are always cheaper sources of sugar that can be used by unscrupulous producers to extend these materials there is a sizeable driving force to “cheat” and make an illicit profit. The route chosen by an unscrupulous producer will depend on the product in question, availability of adulterant and their skill/experience. Most of these products are mainly sucrose and so the use of cane or beet sucrose could be expected.

However, this is not always the case and HFCS is a material which is often chosen instead. This addition will be detectable in maple and coconut flower syrups by distortion of the sugar profile, presence of unusual oligosaccharides by Cap-GC and HPAEC-PAD and dilution of other components plus a shift in the  $\delta^{13}\text{C}$  value.

Birch sap/syrup on the other hand shows low levels of sucrose and roughly equal levels of glucose and fructose, so addition of HFCS to this type of material will show little changes in the sugar levels. However, the addition of this type of material will still be detectable by IRMS & Cap-GC analysis.

Detection of beet and cane sucrose addition to maple sugar/syrup/sap should be detectable by  $^2\text{H}$ -SNIF-NMR<sup>®</sup>. However, due to the much wider distribution pattern for the production of birch sap/syrup/sugar this may make the use of SNIF-NMR less sensitive for this product.

As agave is a CAM plant its  $\delta^{13}\text{C}$  value seen in the sugars of the product are very different from the other products but are similar to sugars derived from cane/corn using the global method. The presence of HFCS in agave is detectable by Cap-GC or by using isotopic methods  $^{13}\text{C}$ -SNIF-NMR.

The origin of sucrose (cane/beet) and glucose syrups (corn/rice) can be achieved using  $^{13}\text{C}$ -IRMS and/or SNIF-NMR<sup>®</sup>.

There are many methods that can be applied to these products, but isotopic methods generally provide one of the best opportunities to detect extension of these products with cheaper sugar sources.

About 5 or more years ago a new screening method was introduced for the analysis of fruit juices using  $^1\text{H}$ -NMR [40]. This method will be discussed in more detail in the chapter on fruit juices. It has also been successfully applied to the analysis of honey to detect sugar additions and it is likely that the same procedure could be applicable to the analysis of agave, maple, birch and coconut flower sugar but this is a “work in progress” for these products.

As unscrupulous suppliers are always looking for new avenues by which they can to extend their products without being detected this will always present a challenge to the analyst. It may be that they will detect new ways to prepare syrups so that they will not carry the markers in use today to detect their addition to these sugar-based products.

As there is significant ecological pressure on suppliers to reduce the new planting of palm trees in Asia which are used for palm oil production, it is highly likely that this may also extend to palm flower sugar/syrup in the future if the popularity of this product grows. In Mexico there is a growing demand for agave pinas for syrup production. However, the supply of pinas is limited at present and there is pressure on prices and availability of the raw material for their production. The availability of these materials must be shared between both syrup and spirit producers (Tequila and Mezcal) and it is likely that raw material prices will remain high while syrup demand remains high/increases.

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